UNIVERSIDADE FEDERAL DO PARANÁ

ODAIR MILIONI DE MEIRA

ANATOMIA COMPARADA E IMPLICAÇÕES FILOGENÉTICAS DA MUSCULATURA ESQUELÉTICA DA CABEÇA DA FAMÍLIA APIDAE *SENSU LATO* (HYMENOPTERA: APOIDEA)

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Orientador: Prof. Dr. Rodrigo Barbosa Gonçalves

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS BIOLÓGICAS (ENTOMOLOGIA) da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ODAIR MILIONI DE MEIRA** intitulada: **ANATOMIA COMPARADA E IMPLICAÇÕES FILOGENÉTICAS DA MUSCULATURA ESQUELÉTICA DA CABEÇA DA FAMÍLIA APIDAE *SENSU LATO* (HYMENOPTERA: APOIDEA)**, sob orientação do Prof. Dr. RODRIGO BARBOSA GONÇALVES, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Esta dissertação é dedicada aos meus pais, Francisco e Maria.

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RESUMO

Estudos recentes indicaram que caracteres derivados da morfologia interna se mostraram filogeneticamente informativos, porém a maioria desses caracteres são relativos as projeções internas do exoesqueleto, relegando aos tecidos moles um papel ainda modesto na sistemática de abelhas. A musculatura da cabeça de Apidae *s. l.* foi estudada minunciosamente apenas para *Apis mellifera* Linnaeus, 1758. O objetivo deste estudo é comparar a musculatura da cabeça em Apidae *s. l*. e avaliar seu potencial como fonte de dados para reconstruções filogenéticas. A musculatura extrínseca da cabeça de *Megalopta sodalis* (Vachal, 1904) é descrita e comparada a 33 espécies pertencentes a seis subfamílias e 26 tribos. Ilustrações detalhadas para as 34 espécies de abelhas analisadas, bem como para uma espécie de Sphecidae e uma de Crabronidae *s. l*. são fornecidas, totalizando 134 imagens. Uma terminologia padronizada para essas estruturas é proposta para resolver inconsistências entre estudos anteriores. Um total de 12 caracteres representando a variação foi otimizada em uma árvore filogenética sumarizada para as espécies examinadas. A principal fonte de informação foi a musculatura relacionada ao aparelho bucal. Quatro caracteres são interpretados aqui como possíveis sinapomorfias para Apidae *s. l*., um como uma possível sinapomorfia de abelhas de língua longa, três possíveis sinapomorfias para Halictinae e uma para Megachilinae. Fêmeas e machos apresentaram morfologia semelhante da musculatura, exceto por uma modificação nos machos de Andreninae. Esta é a primeira abordagem com um uso extensivo da musculatura da cabeça para a sistemática das abelhas fornecendo uma base para futuras investigações filogenéticas em vários níveis taxonômicos. A técnica de baixo custo aqui utilizada pode ser replicada em outros grupos de estudos mesmo com recursos escassos ou sem acesso a equipamentos de alta tecnologia, o que é especialmente relevante em um cenário de modestos investimentos na ciência nacional. Os investimentos são importantes para o desenvolvimento científico e mesmo uma técnica simples como a utilizada neste estudo só é possível devido a investimentos em ciência básica e educação pública gratuita.

Palavras-chave: Abelhas. Morfologia. Filogenia. Dissecação.

ABSTRACT

Recent studies have indicated that characters derived from internal morphology have been shown to be phylogenetically informative, but most of these characters are related to the internal projections of the exoskeleton, relegating soft tissues to a still modest role in the systematic of bees. The head musculature of Apidae *sensu lato* was widely studied only for *Apis mellifera* Linnaeus, 1758. The aim of this study is to compare the head musculature in Apidae *s. l.* and assess its potential as a source of data for phylogenetic reconstructions. The extrinsic musculature of the head of *Megalopta sodalis* (Vachal, 1904) is described and compared to 33 species belonging to six subfamilies and 26 tribes. Detailed illustrations for the 34 species of bees analyzed, as well as for one species of Sphecidae and one of Crabronidae *s. l*. are provided, totaling 134 images. Standardized terminology for these structures is proposed aiming to resolve inconsistencies between previous studies. A total of 12 characters representing the variation was optimized in a summarized phylogenetic tree for the species examined. The main source of information was the musculature related to the labiomaxillary complex. Four characters are interpreted here as possible synapomorphies for Apidae *s. l.*, one as a possible synapomorphy of long-tongued bees, three possible synapomorphies for Halictinae and one for Megachilinae. Females and males showed similar muscle morphology, except for a modification in the males of Andreninae. This is the first approach with an extensive use of the head musculature for the systematic of bees, providing a basis for future phylogenetic investigations at various taxonomic levels. The low-cost technique used here can be replicated in other study groups even with scarce resources or without access to high-tech equipment, which is especially relevant in a scenario of modest investments in national science. Investments are important for scientific development and even a simple technique like the one used in this study is only possible due to investment in basic sciences and free public education.

Keywords: Bees. Morphology. Phylogeny. Dissection.

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1 RESUMO EXPANDIDO

A maioria dos estudos filogenéticos morfológicos com abelhas foca quase exclusivamente em caracteres da morfologia externa e da terminália masculina. Por outro lado, estudos recentes de anatomia interna indicaram a relevância do esqueleto interno como fonte de dados para reconstruções filogenéticas em nível de família e gênero (PORTO et al., 2016a, 2016b; PORTO E ALMEIDA 2019; MEIRA E GONÇALVES, 2018). Os tecidos moles, particularmente a musculatura, não foram amplamente explorados na sistemática das abelhas. Os trabalhos de Roig-Alsina e Michener (1993) investigando as relações filogenéticas das abelhas de língua longa e Alexander e Michener (1995) das abelhas de língua curta são os estudos de anatomia comparada mais abrangentes para o grupo, mas ambos não codificaram quaisquer caracteres relacionados à musculatura esquelética. Plant e Paulus (2016), mais recentemente, apresentam uma revisão com reanálises filogenéticas, mas ainda focados na anatomia externa de escleritos. Para Apoidea, Melo (1999) codificou um caráter relacionado ao músculo interfurcal em sua filogenia, derivado da interpretação de Heraty et al. (1994), e a ausência deste músculo foi considerada uma sinapomorfia para a superfamília.

Os trabalhos focados na anatomia da musculatura da cabeça de Hymenoptera são as investigações de Duncan (1939) com *Vespula pensylvanica* Saussure, 1857, Wolff (1875), Morison (1927), Snodgrass (1942), Urban, (1963), Graf (1965, 1972) e Youssef (1971) com Apidae, Vilhelmsen (1996) e Beutel e Vilhelmsen (2007) com Xyelidae, Zimmermann e Vilhelmsen (2016) com Hymenoptera e Richter et al. (2019) com Formicidae. A variação anatômica da musculatura da cabeça para Apidae pode ser considerada pouco explorada, bem como a proposição de homologias e padronização da terminologia ainda é necessária. Para preencher essa lacuna, este estudo tem como objetivo realizar uma análise de anatomia comparada da musculatura esquelética extrínseca da cabeça de Apidae.

Para isso, fêmeas de 34 espécies pertencentes a seis subfamílias de Apidae, bem como a uma espécie de Crabronidae *s. l.* e uma espécie de Sphecidae, foram examinadas. Adicionalmente, sete machos macrocefálicos e não macrocefálicos pertencentes a quatro subfamílias foram dissecados para verificar possíveis

diferenças sexuais. A maioria dos exemplares foi coletado no estado do Paraná, Brasil, em duas localidades diferentes, no Campus do Jardim Botânico da Universidade Federal do Paraná em Curitiba e no Parque Estadual de Vila Velha em Ponta Grossa exceto *Andrena* sp., *Melitta tricincta* Kirby, 1902, *Dufourea* sp. e *Protosiris mcginleyi* (Shanks, 1986) que foram obtidos por doação. O material testemunho está depositados na Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP).

Todo o material passou por processo de preparação que foi baseado em um protocolo modificado de Dal Molin (2018) contendo as etapas de desidratação e secagem química. Após essa etapa, os espécimes foram dissecados com a utilização de pedaços de lâmina cortante em uma lâmina escavada preenchida com parafina. Foram realizadas seções sagitais e coronal de acordo com os estudos de Youssef (1971). Adicionalmente os tecidos foram corados com a utilização de Lugol 2%. A obtenção das imagens foi realizada em uma Olympus SZ61 (Olympus Corporation, Japão) estereomicroscópica acoplada a uma câmera digital industrial Toupcam UCMOS (Zhejiang, China) com o software ToupView 3.7 (ToupTek Digital, Japão). As imagens obtidas foram então processadas no software Combine ZP 1.0 (Informer Technologies, Inc.) e vetorizadas no software Easy Paint Tool SAI V1.2.5.

Para a análise de morfologia comparada os trabalhos de Snodgrass (1942) e Youssef (1971) sobre *Apis mellifera* Linnaeus, 1758, que estudaram a musculatura cefálica de forma mais abrangente, foram exaustivamente consultados. Para a caracterização topológica dos pontos de origem e inserção muscular e levantamento da nomenclatura utilizada para essas estruturas em Hymenoptera foram consultados os trabalhos de Michener (1944, 2007), Roig-Alsina e Michener (1993), Alexander e Michener (1995), Porto et al. (2016b) e Porto e Almeida (2019), para abelhas, e Duncan (1939), Alam (1951), Matsuda (1957, 1965), Vilhelmsen (1996), Vilhelmsen e Beutel (2007) e o catálogo Hymenoptera Anatomy Ontology (HAO, 2021). Ao longo do texto, a escolha de uso foi pelos termos apresentados no catálogo HAO (2021). Em casos onde a correspondência de termos junto ao HAO não foi possível, a terminologia apresentada por Youssef (1971) foi adaptada para o padrão apresentado no HAO em forma de propostas de inclusão para cada termo contendo descrição, sinônimos e comentários adicionais quando pertinente.

Toda a variação encontrada foi avaliada para a construção de caracteres filogenéticos seguindo as recomendações de Sereno (2007), então compilada em forma de matriz de dados para as 36 espécies analisadas, e otimizada sobre uma árvore sumário a partir do estudo de Hedtke et al. (2013). A topologia dessa árvore foi editada no *software* Mesquite (MADISON E MADISON, 2019) de forma a conter apenas as espécies aqui analisadas segundo a sua classificação taxonômica e os caracteres construídos foram analisados primariamente com a opção de otimização não ambígua e alternativamente com as opções de otimização ACCTRAN e DELTRAN no *software* Winclada (NIXON, 2002)

No presente estudo, a musculatura extrínseca da cabeça das espécies de abelhas foi analisada em detalhes e uma terminologia padronizada é fornecida. Também é apresentada uma proposta de integração de seis músculos que não constam no catálogo HAO (2021). São ilustrados pela primeira vez os músculos da cabeça de espécies de cinco subfamílias de Apidae em um total de 134 vetorizações, aumentando o conhecimento da morfologia do grupo. Foram propostos 12 caracteres filogenéticos, sendo quatro novas sinapomorfias possíveis para a família. Entre as subfamílias, Halictinae apresentou a morfologia mais peculiar provavelmente associada à conformação do seu aparato labiomaxilar. Apinae foi a subfamília com a morfologia mais heterogênea entre as espécies, possivelmente derivada da idade e diversificação deste clado ou do efeito da maior amostragem.

Algumas das características estudadas mostraram uma alta variabilidade e podem ainda ser comprovadas como úteis para outros níveis taxonômicos. Além disso a terminologia padronizada proposta aqui deve auxiliar em futuros estudos comparativos para abelhas e grupos filogeneticamente próximos. Em linhas gerais este estudo apresenta a primeira análise comparativa da musculatura extrínseca da cabeça das abelhas. A técnica de baixo custo aqui utilizada pode ser replicada em outros grupos de estudos mesmo com recursos escassos ou sem acesso a equipamentos de alta tecnologia, o que é especialmente relevante em um cenário de investimentos modestos na ciência nacional. Os investimentos são importantes para o desenvolvimento científico e mesmo uma técnica simples como a utilizada neste estudo tem por trás um alto investimento em ciência básica e educação pública gratuita.

2 INTRODUCTION

Bees are a monophyletic group (ALEXANDER AND MICHENER, 1995; MELO, 1999; OHL AND BLEIDORN, 2006; DEBEVEC et al., 2012) with 20,507 recorded species (ASCHER AND PICKERING, 2021). The most widely used classification for bees recognizes seven families (MICHENER, 2007), however this study follows the single-family classification of Melo and Gonçalves (2005), with bees divided into seven subfamilies, Andreninae, Apinae, Colletinae, Halictinae, Megachilinae, Melittinae, and Stenotritinae. The adoption of this classification is due to the fact that it provides a formal classification name for the bees as a whole and additionally makes their classification more compatible with the higher-level classification system employed for the aculeate Hymenoptera. The family belongs to Apoidea, and the phylogenetic relationships among the lineages of this superfamily are still controversial. Melo (1999) presented morphological support to the Apidae + Crabronidae, however, recent studies (OHL AND BLEIDORN, 2006; OHL AND SPAHN, 2010; DEBEVEC et al., 2012; PETERS et al., 2017; SANN et al., 2018) have found that Crabronidae is not monophyletic and postulate Pemphredoninae (PETERS et al., 2017) or Ammoplaninae (SANN et al., 2018), as sister-group of the bees.

The phylogenetic relationships among bee subfamilies based on molecular studies are mostly stable (Fig. 1), with all subfamilies recovered as monophyletic and Melittinae considered as the sister-group to the remaining bees (DANFORTH et al., 2006; HEDTKE et al., 2013, BRANSTETTER et al., 2017, HUSEMANN et al., 2021). The long-tongued bees, Apinae and Megachilinae, are considered a monophyletic group, while Andreninae, Colletinae, Halictinae and Stenotritinae (the short-tongued bees except Melittinae) are another clade recognizable by the hemicryptic mid coxae (MICHENER, 1981). This knowledge about bee phylogeny is important to interpret character evolution, presently those derived from musculature.

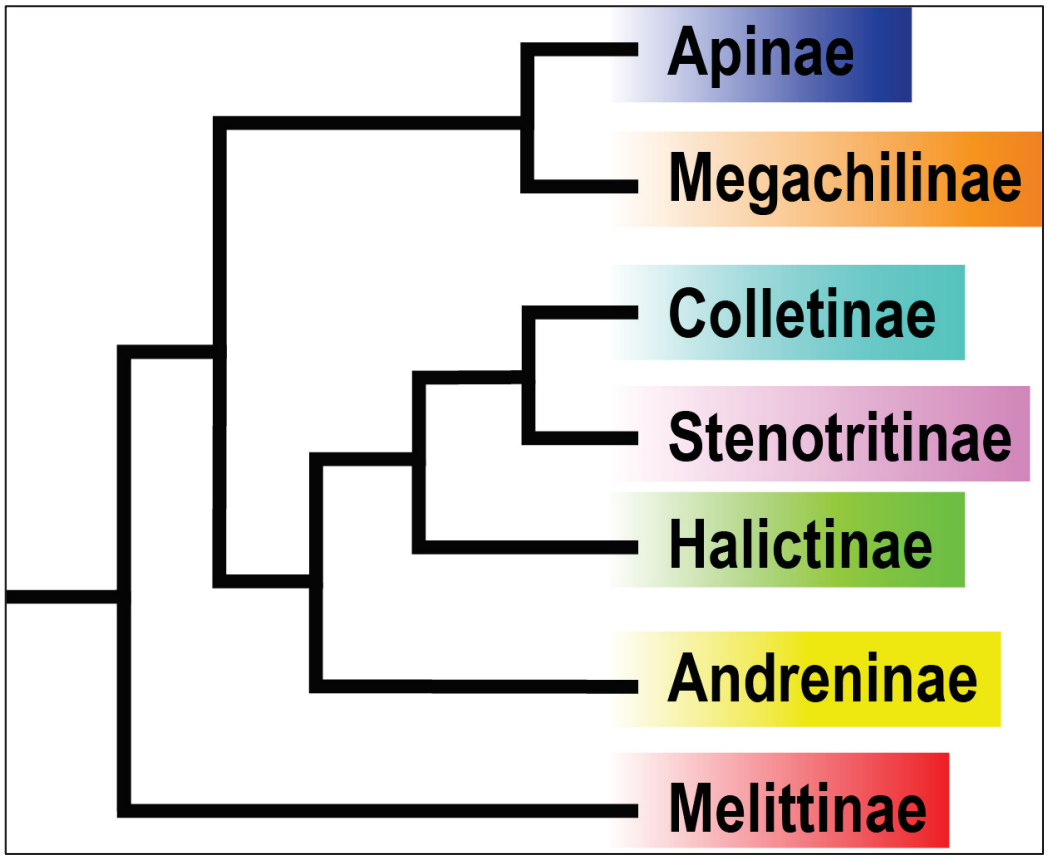

FIGURE 1. THE PHYLOGENETIC RELATIONSHIPS AMONG APIDAE SUBFAMILIES. MODIFIED OF HEDTKE ET AL. (2013).

Most morphological phylogenetic studies within bees are largely focused on characters of external morphology and male terminalia (genital capsule and sterna). Recent studies based on internal anatomy indicated the relevance of the internal skeleton as a data source to phylogenetic reconstructions at family- and genus -level (PORTO et al., 2016a, 2016b; MEIRA AND GONÇALVES, 2018; PORTO AND ALMEIDA, 2019). Soft tissue, particularly musculature, has not been explored in bee systematics at the same intensity. The works of Roig-Alsina and Michener (1993) investigating the phylogenetic relationships within the long-tongued bees and Alexander and Michener (1995) of short-tongued bees remains the most comprehensive comparative anatomy studies for the group. However, both studies did not consider any characters related to skeletal musculature. Plant and Paulus (2016), more recently, integrate a review with phylogenetic reanalysis, but still focused on the external anatomy of sclerites. For the superfamily Apoidea, Melo (1999) coded a character related to the interfurcal muscle derived from the interpretation of Heraty et

al. (1994), and the absence of this muscle is considered a synapomorphy for the superfamily. Recently, musculature was used by Zimmerman and Vilhelmsen (2016) to investigate the sister-group of Aculeata, the authors included *Pison* sp. (Crabronidae) in their analysis.

Internal morphological studies have gained recent attention due to the use of microtomography and other high-tech equipment. For examples that include musculature, there is Wipfler (2011) for Grylloblattodea, Zimmerman and Vilhelmsen (2016) for Hymenoptera, and Richter et al. (2019) for Formicidae. Simple dissection techniques have a long history of use, including for phylogenetic interpretations, but are considered strongly dependent on the skill and experience of the researcher (Wipfler et al., 2016). On the other hand, techniques with high-cost equipment and maintenance are barriers to emergent research groups and bring to morphology the same dependency to “wealthy” laboratories and researchers that are observed in molecular systematics studies.

The contributions focused on the anatomy of Hymenoptera head musculature are the investigations of Duncan (1939) with *Vespula pensylvanica* Saussure, 1857 (Vespidae), Wolff (1875), Morison (1927), Snodgrass (1942), Urban (1963), Graf (1965, 1972) and Youssef (1971) with Apidae*,* Vilhelmsen (1996) and Beutel and Vilhelmsen (2007) with Xyelidae, Zimmerman and Vilhelmsen (2016) for Hymenoptera and Richter et al. (2019) for Formicidae. Concerning Apidae, the anatomical variation of the musculature is mostly unexplored and outdated, the last study is from 1972. Hence, a standardized study of bee head musculature can increase knowledge about its evolution and can provide a basis for further studies at other taxonomic levels.

3 OBJECTIVES

The main objective of this thesis is to provide a comparative anatomical analysis of the head extrinsic skeletal musculature of Apidae using a simple dissection technique.

The specific objectives are: (1) to propose a terminology standardization for bee head musculature based on previous works with *Apis mellifera* Linnaeus, 1758 and recent studies with Hymenoptera; (2) to explore the link between muscle and skeleton variation; (3) to elaborate homology assessments; (4) to optimize the character states on bee phylogenetic tree to assess the phylogenetical potential of this data source; (5) to evaluate possible differences in the head musculature between females and males.

4 METHODOLOGY

4.1. TAXONOMIC SAMPLING

Females from 34 species belonging to six subfamilies of Apidae were examined (Table 1). The selection criterion of the internal group consisted of sampling species from different subfamilies and tribes aiming at a high phylogenetic variation and also the accessibility to fresh material allowing a high-quality analysis. For the outgroup, one species from Crabronidae *s.l*. and one from Sphecidae were included. Most of the examined specimens, except for *Andrena* sp. (from Germany), *Dufourea* sp. (from USA), *Melitta tricincta* Kirby, 1802 (from Germany) and *Protosiris mcginleyi* (Shanks, 1986) (from USA) obtained from donation, were collected in the state of Paraná, Brazil, in two different localities, Campus of the Universidade Federal do Paraná (Campus Jardim Botânico) in Curitiba, and Vila Velha State Park (Parque Estadual de Vila Velha) in Ponta Grossa. In addition to females, seven macrocephalic and non-macrocephalic males belonging to four subfamilies were examined to investigate the differences between sexes and dimorphic males. Vouchers are deposited in Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP).

TABLE 1. CLASSIFICATION OF STUDIED SPECIES AND SAMPLED SEXES.

FAMILY	SUBFAMILY	TRIBE	SPECIES	SEX
Apidae	Andreninae	Andrenini	*Andrena* sp.	♀
		Calliopsini	*Callonychium (Callonychium) petuniae* Cure & Wittmann, 1990	♀,♂
		Protandrenini	*Psaenythia annulata* Gerstaecker, 1868	♀,♂
		Protandrenini	*Psaenythia bergii* Holmberg, 1884	♀,♂
		Protandrenini	*Anthrenoides meridionalis* (Schrottky, 1906)	♂
		Protandrenini	*Rhophitulus* sp.	♀
	Apinae	Apini	*Apis mellifera* Linnaeus, 1758	♀
		Bombini	*Bombus (Fervidobombus) morio* (Swederus, 1787)	♀
		Centridini	*Centris (Hemisiella) tarsata* Smith, 1874	♀
		Emphorini	*Melitoma segmentaria* (Fabricius 1804)	♀
		Ericrocidini	*Mesoplia* sp.	♀
		Eucerini	*Melissoptila aureocincta* Urban, 1968	♀
		Eucerini	*Thygater (Thygater) analis* (Lepeletier, 1841)	♀
		Euglossini	*Eulaema (Apeulaema) cingulata* (Fabricius, 1804)	♂
		Meliponini	*Melipona (Melipona) quadrifasciata* Lepeletier, 1836	♀
		Meliponini	*Scaptotrigona bipunctata* (Lepeletier, 1836)	♀
		Osirini	*Protosiris mcginleyi* (Shanks, 1986)	♀
		Tapinotaspidini	*Arhysoceble* sp.	♀
		Tetrapediini	*Tetrapedia diversipes* Klug, 1810	♀
		Xylocopini	*Ceratina (Rhysoceratina) stilbonota* Moure, 1941	♀
		Xylocopini	*Xylocopa (Neoxylocopa) augusti* Lepeletier, 1841	♀
	Colletinae	Colletini	*Colletes petropolitanus* Dalla Torre, 1896	♀
		Diphaglossini	*Ptiloglossa* sp.	♀
		Hylaeini	*Hylaeus* sp.	♀
		Neopasiphaeini	*Tetraglossula anthracina* (Michener, 1989)	♀
	Halictinae	Augochlorini	*Augochlora (Augochlora) daphnis* Smith, 1853	♀,♂
		Augochlorini	*Megalopta sodalis* (Vachal, 1904)	♀
		Caenohalictini	*Agapostemon (Notagapostemon) semimelleus* Cockerell, 1900	♀
		Halictini	*Dialictus opacus* (Moure, 1940)	♀
		Rophitini	*Dufourea* sp.	♀
	Megachilinae	Anthidiini	*Moureanthidium paranaense* Urban, 1995	♀,♂
		Megachilini	*Coelioxys* sp.	♀
		Megachilini	*Megachile (Moureapis) apicipennis* Schrottky, 1902	♀
	Melittinae	Melittini	*Melitta tricincta* Kirby,1802	♀
Crabronidae *s.l.*	Crabroninae	Trypoxylini	*Trypoxylon (Trypargilum) lactitarse* Saussure, 1867	♀
Sphecidae	Sceliphrinae	Podiini	*Podium* sp.	♀

4.2. DISSECTION, OBSERVATION AND IMAGING

The specimens were initially conditioned in > 70% ethanol and before starting the dissection, the head was separated from the body with the aid of entomological forceps, forcing it in the anteroposterior direction in the region between the head and the mesosoma. The antennae and the labiomaxillary tube were not separated from the head to prevent rupture of their extrinsic musculature, especially that connected to the tentorium. For the specimen preparation, the protocol of chemical drying of small

arthropods (DAL MOLIN, 2018) was implemented with minor modifications as described below.

4.2.1. Chemical drying protocol modified from Dal Molin (2018)

STEP 1. Dehydration, with successive baths in sequentially high graduations of ethanol as follows:

- 30 minutes in ethanol 75%;
- 30 minutes in ethanol 80%;
- 30 minutes in ethanol 90%;
- 30 minutes in ethanol 96%;
- 30 minutes in ethanol 99.9%;

After this sequence, the specimen is immersed in 100% ethanol for one hour.

STEP 2. Drying the specimen in successive baths as follows:

- 1 hour in a 1:1 100% ethanol and isoamyl acetate solution.
- 1-2 hours (1 hour if less than 2mm and 2 hours if greater than 2mm) in pure isoamyl acetate.
- Final bath in pure isoamyl acetate until it evaporates.

After the chemical drying the specimen can be stored in a microcentrifuge tube filled with absolute ethanol or bi-distilled glycerin.

4.2.2. Dissection

The muscle dissection is based upon the study of Youssef (1971) with minor modifications. A thin layer of paraffin is heated with a Bunsen burner in an excavated slide until it becomes liquid. For the obtaining of the sagittal sections, with the paraffin wax in a liquid state, the dried specimen is placed with the frontal region of the head directly in contact with the paraffin so that the occiput is facing upwards (Fig. 2B), for the coronal sections the position of the dried specimen is inverted, therefore the occiput is directly in contact with the paraffin and the frontal region of the head is facing

upwards. The specimen is left in the desired position until the paraffin solidifies again, holding the structure firmly. It is recommended that the specimen be laid on the excavated slide at the moment of paraffin solidification, defined as when the edges of the liquid paraffin begin to solidify, this process starts at the edges and moves towards the center giving enough time to place the specimen before the full solidification. This procedure is indicated to prevent dissolved paraffin from entering the head capsule cavity through the opening of the occiput or the mouth.

With the specimen firmly positioned on the excavated slide, the sagittal sections were carried out with a razor blade. After dissection, the sections can be stored in containers such as microcentrifuge tubes containing bi-distilled glycerin or follow the observation step. The following sections were carried out:

- Sagittal section 1 (S1): following the line of the internal side of the compound eye until the vertex (Fig. 2A-B). This cut usually allows the visualization of the mandibular muscles and, in some groups, of the muscles of the gena and occiput.
- Sagittal section 2 (S2): following the line of the internal side of the mandible until the vertex (Fig. 2A-B). This cut allows the observation of the genal, premental, and antennal muscles.
- Sagittal section 3 (S3): following the internal line of the antennal foramen (Fig. 2A-B). This cut allows the visualization of a considerable number of muscles, especially the antennal, maxillary, labial, frontal, clypeal, cibarial, labral and pharyngeal muscles.
- Sagittal section 4 (S4) following the median line of the head (Fig. 2A-B). This cut allows the visualization of muscles that are hidden in the other cuts.
- Coronal section (C1), also called as frontal section, cuts the head from front to back, separating the anterior and posterior regions (Fig. 2B). It is applied for the visualization of specific muscle groups, as the anterior cibarial wall muscles.

The dissection of one species is done on average in eight hours of work, taking into account all the cuts and fine adjustments with the removal of sclerites for the

display of all the muscle groups studied here. This average time increases substantially if the sample is very small due to the difficulty of handling.

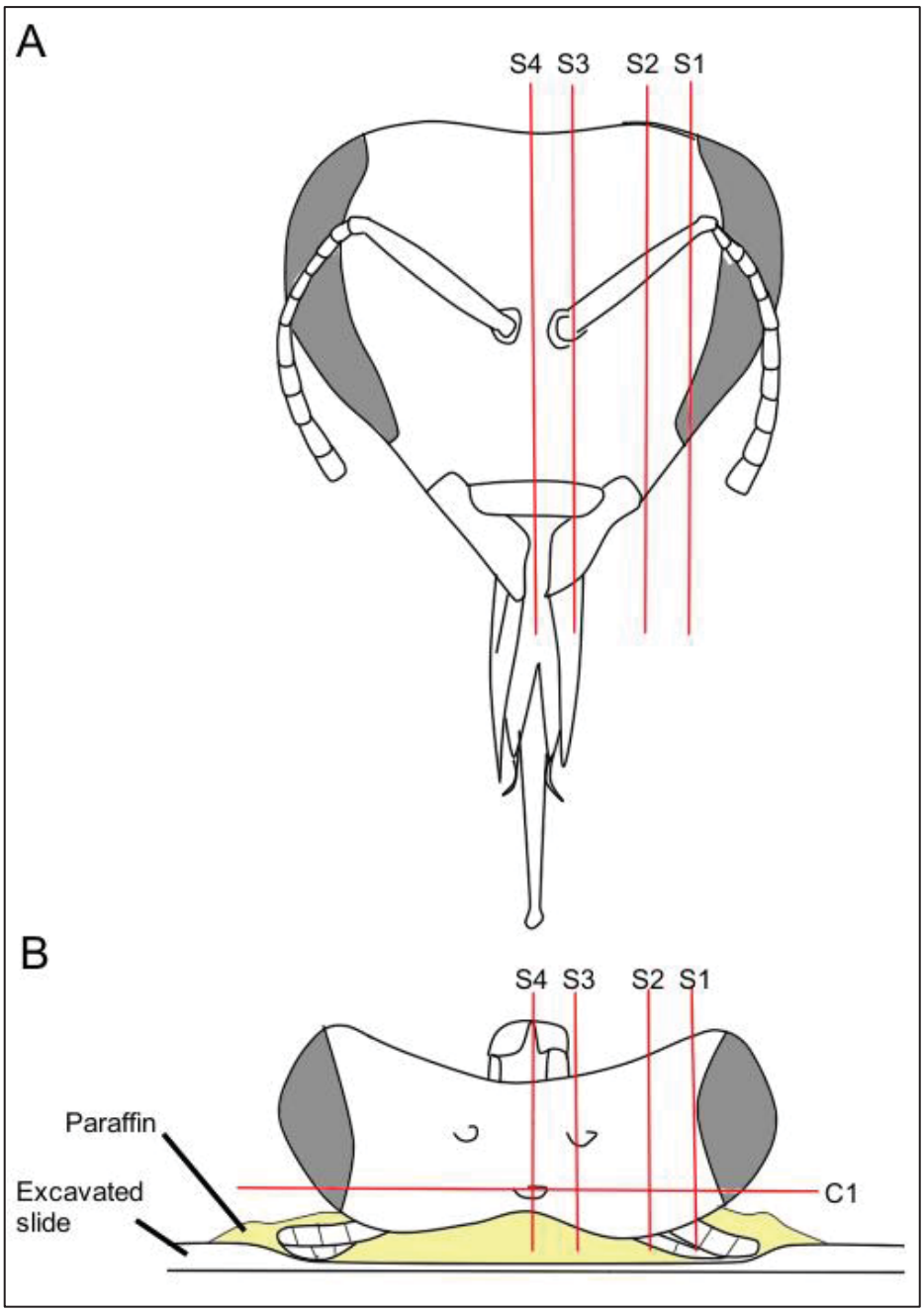

FIGURE 2. THE SAGGITAL AND CORONAL CUTS APPLIED HERE. A. ANTERIOR VIEW INDICATING SAGGITAL SECTIONS; B. DORSAL VIEW INDICATING ORIENTATION FOR THE DISSECTION STEP ON SLIDE, SAGGITAL AND CORONAL SECTIONS; S1. SAGITTAL SECTION 1; S2. SAGITTAL SECTION 2; S3. SAGITTAL SECTION 3; S4. SAGITTAL SECTION 4; C1. CORONAL SECTION.

4.2.3. Staining and observation

Staining with 2% iodine is recommended after the dissections to facilitate muscle identification. The duration of this procedure is variable and it is suggested that the sample be removed from the microcentrifuge tube at short intervals to measure the degree of staining. If the specimen is stored in bi-distilled glycerin, it must be transferred to a Petri dish containing absolute ethanol for a few minutes so that the glycerin is dissolved.

After staining, the specimen can be observed in Petri dishes containing bi-distilled glycerin or absolute ethanol. Water is not recommended as it would tend to hydrate the muscles, reversing the process of the chemical drying protocol (4.2.1). These liquids allow specific variations for observation depending on the magnification, such as greater or lesser mobility of the sample, transparency and staining duration. It is important to note that this staining is not permanent, so it must be repeated with each new observation or as soon as the coloration begins to disappear. After examination, the dissections were stored in microcentrifuge tubes filled with absolute ethanol and deposited in Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP).

4.3. IMAGE ACQUISITION

The structures were analyzed with incident/transmitted light on an Olympus SZ61 (Olympus Corporation, Japan) stereomicroscopic coupled with a Toupcam industrial digital camera UCMOS camera (Zhejiang, China) with the ToupView 3.7 software (ToupTek Digital, Japan) on an excavated slide with the aid of intimate water-based lubricating gel so that the structures do not move excessively. The stack of images obtained with the focus stacking procedure is processed in the Combine ZP 1.0 software (Informer Technologies, Inc.). This software takes the stack of images with a different focus and combines them into a single multifocal image. The processed multifocal image is then vectorized in the Easy Paint Tool SAI V1.2.5 software and to distinguish each of the analyzed muscles, unique colors and numbers for each muscle were applied to each illustration. The complete vectorization of images for one species

takes on average 6-8 hours of continuous work, considering the acquisition of all images and illustrations.

4.4. TOPOLOGICAL CHARACTERIZATION AND TERMINOLOGY

The skeletal muscles of insects are bundles of elongate, multinucleate cells called muscle fibers. Besides skeletal muscles, insects also contain visceral and cardiac muscles (JOSEPHSON, 2009). In general, the musculature can be distinguished in two categories; those with origin and insertion located inside an appendix (intrinsic) such as the antennae or legs, and those with origin and / or insertion points located outside a specific appendix (extrinsic). Beutel et al. (2014) listed 83 head muscles, including those intrinsic to the antennae and the mouthparts. This study analyzed only the extrinsic musculature of these appendages.

For the comparative analysis, the honeybee comprehensive studies of Snodgrass (1942) and Youssef (1971) were exhaustively consulted for muscle interpretation, but also works with more specific muscles for Apidae were consulted (WOLFF, 1875; MORISON, 1927; URBAN, 1963; GRAF, 1965, 1972). For the topological characterization of the muscle attachment, the studies of Michener (1944 and 2007), Roig-Alsina and Michener (1993), Alexander and Michener (1995), Porto et al. (2016b), Porto and Almeida (2019) were consulted. Additionally, the pertinent literature available related to the extrinsic musculature of the head in several groups of Hymenoptera (DUNCAN, 1939; ALAM, 1951; MATSUDA, 1957, 1965; VILHELMSEN, 1996; BEUTEL AND VILHELMSEN, 2007) and the HAO (2021) were consulted.

For head musculature ontology the works of Wolff (1875), Morison (1927), Duncan (1939), Snodgrass (1942), Alam (1951), Matsuda (1957, 1965), Urban (1963), Graf (1965, 1972), Youssef (1971), Vilhelmsen (1996), Beutel and Vilhelmsen (2007), Wipfler et al., (2011), Beutel et al., (2014), Zimmermann and Vilhelmsen (2016) and the HAO (2021) were consulted. In order to standardize terminology, the terms presented in the HAO (2021) were carefully compared with the literature on bees and closely related groups.

The Table 2 shows that the nomenclature system of cephalic muscles in Hymenoptera is usually based on muscle function or topology. It can be argued that the classification system based on the muscle function is more stable than the one based on topology, however, this system seems to be misleading in relation to muscle homology based on similarity. Traditionally, to determine the function of each muscle the main artifice is the micromanipulation of the structure and the observation of its movement, however, several times within smaller muscles, this can only be determined by deduction. Table 2 also shows that different functional names were given by different authors for the same muscles of *A. mellifera*. Furthermore, it is difficult and often impossible identify the corresponding muscles based only on functionality. For example, a muscle can be an abductor in one species, but its homologue in other taxa may be a protractor only because a slight change in the point of origin or insertion, or both. The geno-cardinal muscle of *A. mellifera* was designated by Snodgrass (1942) as "cardinal protractor of proboscis" or "promoter of the maxilla "; whereas it is an “extensor” for Morison (1927) and a "productor" in *Vespula pensylvanica*, according to Duncan (1939). Giving this, the nomenclature system based on the muscle topology will be adopted here. Therefore, names applied to muscles in this study are composed of two terms separated by a hyphen, where the first term refers to the structure of the muscle origin or the fixed attachment point and the second term refers to the muscle insertion point or movable attachment point. For muscles that were not found in HAO (2021), the terms used for these muscles in the study of Youssef (1971) were adapted to the terminological standard presented by HAO (2021) and a propose of formal inclusion of these muscles in the HAO (2021) was also presented (Section 5.3).

4.5. CHARACTER CODING AND OPTIMIZATION

A comparative study of the skeletal musculature of the head was carried out to propose character statements. All the characters proposed in this analysis were derived from the muscle morphology present in the cephalic capsule of adult specimens. The character state matrix derived from the present study was made using the Winclada software version 1.00.08. Each character was treated as a grouping hypothesis (taxic homology *sensu* Patterson (1982); primary homology *sensu* de Pinna

(1991)). During the construction of the characters, it was sought to respect the independence and hierarchy of the characters, as well as the exclusive conditions of each character (de Pinna, 1991; Sereno, 2007). Only one character with multiple states was proposed, in most cases contingent construction by logical dependency was applied (Brazeau, 2011).

For the coding, suggestions of Sereno (2007) were followed in order to increase the objectivity and repeatability of this study. Each character was described by two sentences. The first is related to the structure, where it is located and what is varying. The second sentence consists of the states of this character, and each state is described by a term and listed as 0, 1 and 2 and so on. This structure was followed for all characters that are listed in the character list (5.4).

The character optimization was performed on a summary tree derived from the phylogenetic tree proposed by Hedtke et al. (2013). The topology of the summary tree was edited in Mesquite (MADISON AND MADISON, 2019) with the studied species placed according to their phylogenetic or taxonomic affinities with those from Hedtke et al. (2013). Character transformations were primarily analyzed under unambiguous optimization and characters with ambiguous distribution were alternatively analyzed with the ACCTRAN and DELTRAN optimizations in Winclada software version 1.00.08.

5 RESULTS

5.1. TERMINOLOGY

Different authors present alternative methods for proposing muscle terminology as demonstrated in Table 2. Previous works, for example Wolf (1875), Morison (1927) and Snodgrass (1942), use a nomenclatural system based on the function of the muscle. For example, the muscle that originates in the front and inserts into the labrum tormae in Morison's (1927) study is called levator labri, while for Snodgrass (1942) it is the adductor of the labrum. Subsequent works began employing terminology based on the topology of muscle attachments, that is, the name would be a junction of the fixed and movable attachment points. Following the previous example, for Matsuda (1965) the posterior fronto-labral muscle and, for Youssef (1971) the musculus fronti-labrualis. Although some authors analyzed the same species and using very similar nomenclatural systems, the nomenclature differed substantially and is still not unified to this day. In this work, a standardized terminology is proposed (Table 3) based on the muscle topology criterion and adapting it to the terminological standard adopted by the HAO catalog (2021).

5.2. EXTRINSIC SKELETAL MUSCULATURE OF THE HEAD OF APIDAE *S.L.*

The extrinsic skeletal musculature of the bee head can be divided into nine groups following the classification of Youssef (1971) based on the insertion points of the musculature: labral muscles, epipharyngeal muscles, mandibular muscles, antennal muscles, maxillary muscles, labial muscles, pharyngeal plate muscles, muscles of the anterior cibarial wall and pharyngeal muscles. This classification will be used here. Most studies of head musculature are based on the model species *A. mellifera*, but to provide an alternative to the species usually used and to illustrate the variation presented by these structures a detailed description and illustration (Figs. 3-6) of the Halictinae *Megalopta sodalis* (Vachal, 1904) was presented here. This species has a derived musculature associated with the remarkable shape of the Halictinae labiomaxillary tube, which is possibly different from other Hymenoptera.

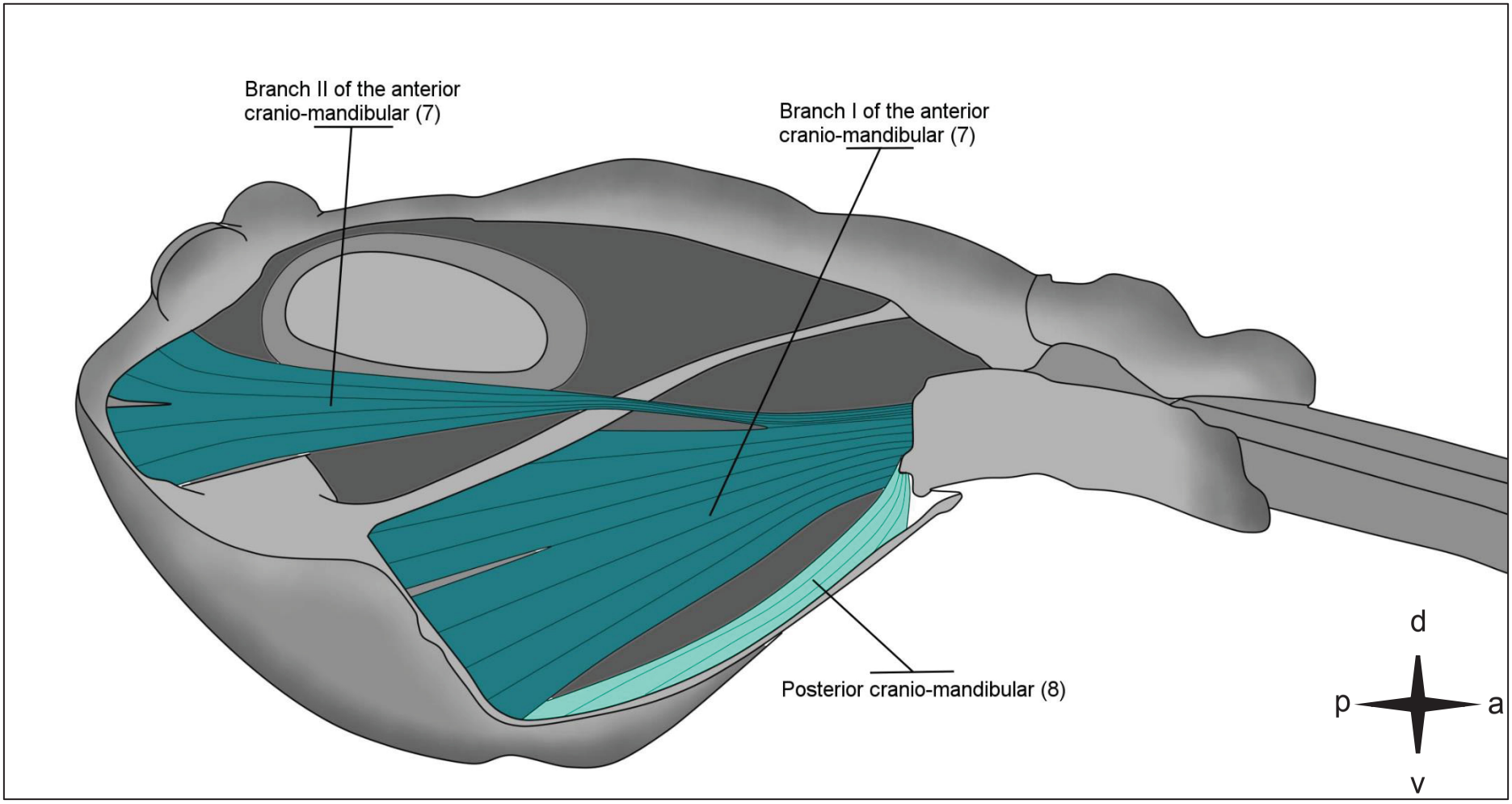

FIGURE 3. FIRST SAGGITAL SECTION (S1) OF FEMALE *MEGALOPTA SODALIS* (VACHAL, 1904). MANDIBULAR MUSCLES. NUMBERS ACCORDING TO TABLE 3.

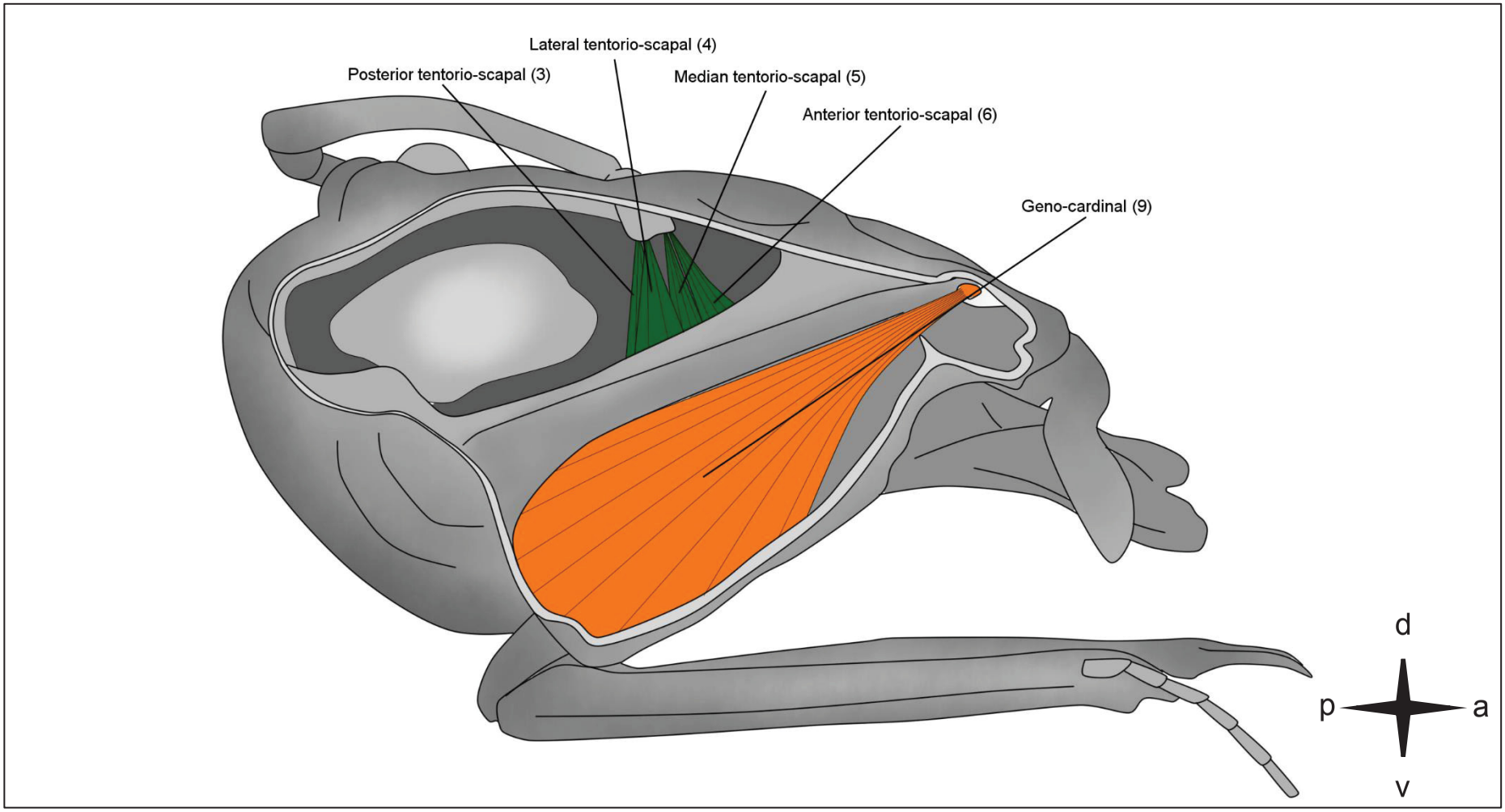

FIGURE 4. SECOND SAGGITAL SECTION (S2) OF FEMALE *MEGALOPTA SODALIS* (VACHAL, 1904). ANTENNAL AND MAXILLAR MUSCLES. NUMBERS ACCORDING TO TABLE 3.

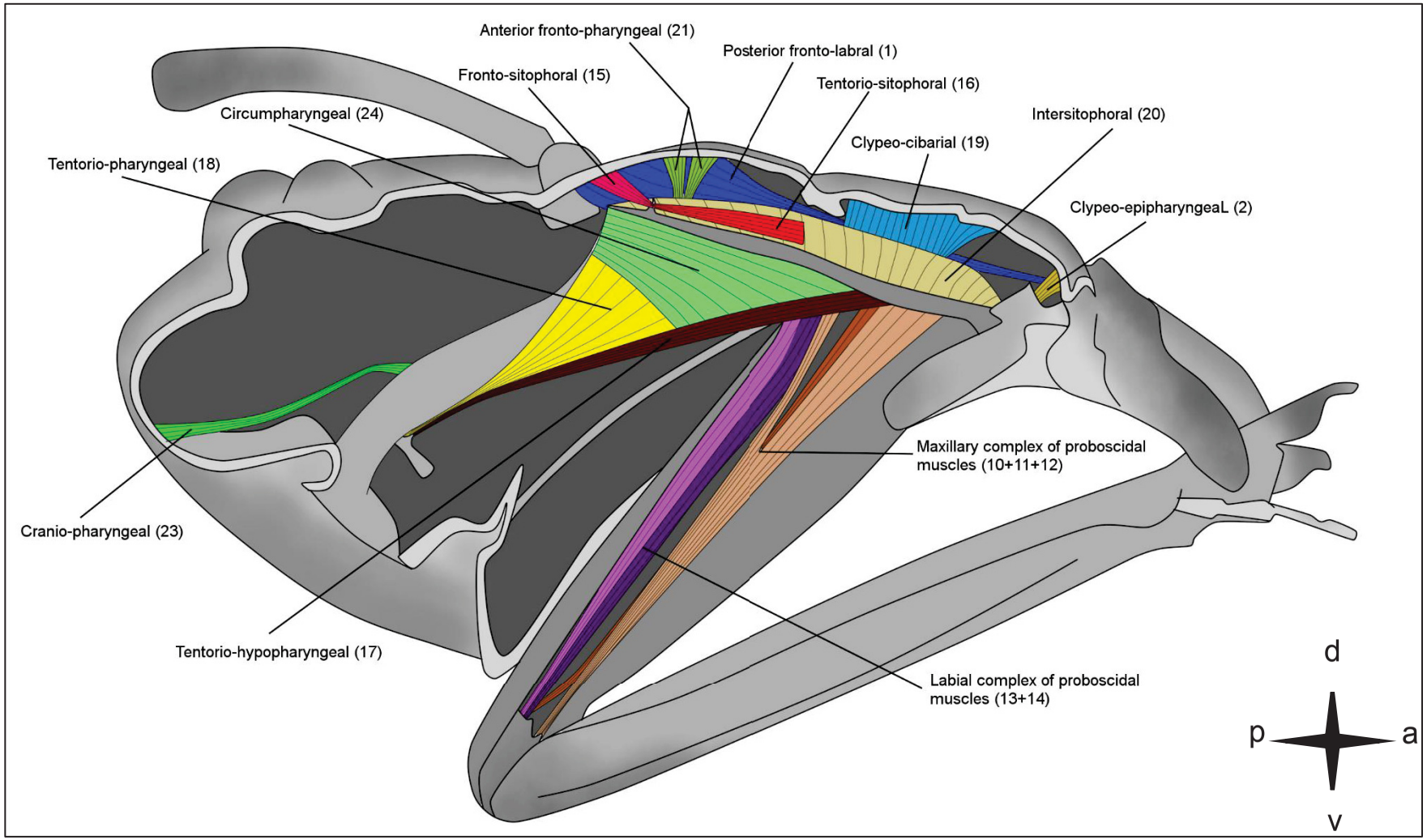

FIGURE 5. THIRD SAGGITAL SECTION (S3) OF FEMALE *MEGALOPTA SODALIS* (VACHAL, 1904). LABRAL, EPIPHARYNGEAL, MAXILLAR, LABIAL, PHARYNGEAL PLATE, ANTERIOR CIBARIAL WALL AND PHARYNGEAL MUSCLES. NUMBERS ACCORDING TO TABLE 3.

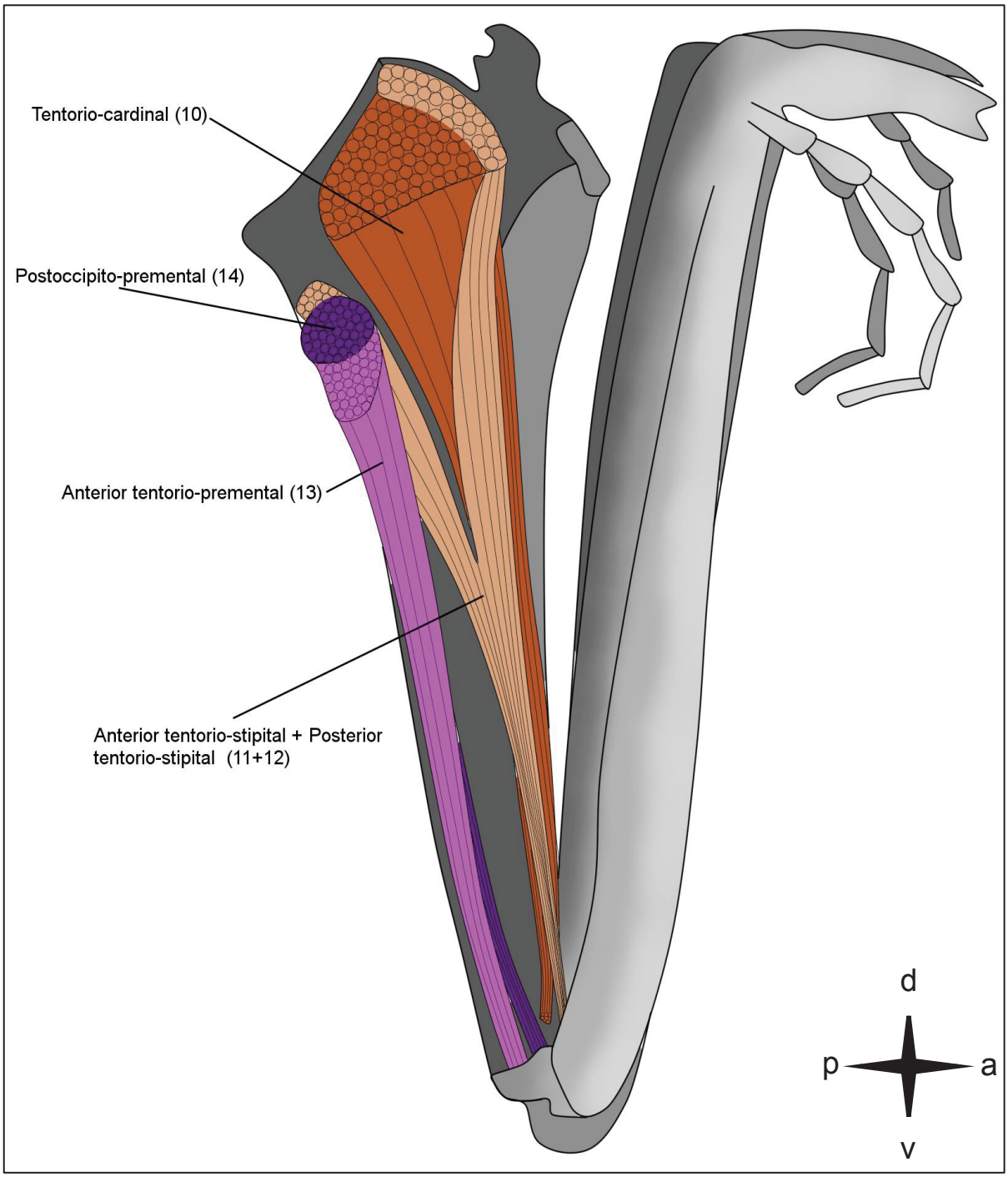

FIGURE 6. DISSECTION OF THE ANTERIOR PART OF THE LABIOMAXILLARY TUBE OF FEMALE *MEGALOPTA SODALIS* (VACHAL, 1904). MAXILLARY AND LABIAL MUSCLES. NUMBERS ACCORDING TO TABLE 3.

TABLE 2. MORPHOLOGICAL TERMINOLOGY AND ONTOLOGY IDENTIFIERS OF THE BEE HEAD MUSCULATURE. FIRST COLUMN NUMBERED TO FOLLOW THE CORRESPONDENCE OF MUSCLE TERMINOLOGY. NUMBERS AND CODES ALONG MUSCLE NAMES ACCORDING TO NUMBERING OF ORIGINAL STUDIES.

	Wolf, 1875	Morison, 1927	Duncan, 1939	Snodgrass, 1942	
1	-	Levator labri	-	Adductor of labrum	1
2	Levator veli planti	Levator veli planti	Clypeo-dilator of the mouth	Retractor or levator of	25
3	-	Musculi antennae	External antennal depressor	Extrinsic muscles of antenna	2
4	-		Internal antennal depressor		3
5	-		External antennal levator		4
6	-		Internal antennal levator		5
7	-	Extensor mandibulae	Mandibular abductor	Abductor of the mandible (a)	8
8	-	Flexor mandibulae	Mandibular adductor	Adductor of the mandible (b)	9 (a, b)
9	Protractor cardinis vel	Extensor maxillae externus	Productor of the cardo	Cardinal protractor of proboscis	10
10	Maxillae inferior internus	Extensor maxillae internus	Extensor of the maxilla	Adductor of the maxilla	11
11	Retractor maxillae inferioris major	Flexor maxillae major	Flexor of stipes	Adductor of maxilla	13
12	Retractor maxillae inferioris minor	Flexor maxillae minor	-	Adductor of maxilla	12
13	Retractor linguae longus	Retractor ligulae superior	Flexor of paraglossa	Anterior adductor of the labium	17
14	Protractor labii inferior vel menti	Protractor labii	Posterior labial adductor	Posterior adductor of labium	18
15	-	Retractor pharyngis superior	-	Retractor of oral plate	33
16	Retractor pharyngis inferior	Protractor pharyngis	Lateral pharyngeal muscle	Protractor of oral plate	32
17	-	Lateral dilator	Posterior dilator of anterior pharynx	Posterior contractor of pharynx	37
18	-	Lateral compressor	Ventral dilator of anterior pharynx	Parietal contractor of the pharynx	38
19	-	Dilator pharyngis	Dilator buccalis	Dilators of the suctorium	28
20	-	Pharyngeal compressor	Pharyngeal dilator of the mouth	Compressors of the suctorium	31
21	-	Dilator pharyngis	Frontal dilator of anterior pharynx	Precerebral dilators of pharynx	34
22					35
23	-	-	Dorsal dilator of posterior pharynx	Postecerebral muscle of pharynx	36
24	-	-	Intrinsic muscle of the pharyngeal diverticulum	Constrictors of the pharynx	39

CONTINUATION TABLE 2.

	Alam, 1951		Matsuda, 1957		Urban, 1963	Graf, 1965	
1	Abductor of the labrum	1	Frons-epipharynx	1	Adutor labral		
2	retractor of the epipharynx	2	Labrum epipharynx	3,4			
3	External depressor	7	Tentorium-antenna		Músculos da antena		
4	Internal depressor	8					
5	External levator	6					
6	Internal levator	5					
7	Abductor of the mandible	3	Abductor of the mandible	22	Abdutor mandibular		
8	Adductor of the mandible	4	Adductor of the mandible	23	Adutor mandibular		
9	First protractor of the maxillae	22			Protrator cardinal da probóscide	Protrator cardinal da probóscide	9
10	Second protractor of the maxillae	23	tentorium-cardo	25		Adutor médio da maxila	11
11	Flexor of maxillae	24	tentorium-lacinia	26	Protratores estipitais da probóscide	Adutor anterior da maxila	10
12	Second flexor of maxilae	26				Adutor posterior da maxila	12
13	Posterior adductor	14	Tentorium-prementum	36	Retrator labial da probóscide	Retrator labial da probóscide	1
14	Anterior adductor	13	Tentorium-glossa		Protrator do lábio	Protrator do lábio	2
15	Frontal dilator of pharyngeal diverticula	35	Frons-stomodaeum	9			
16	Tentorial dilator of pharyngeal diverticula	34	Tentorium-stomodaeum	12			
17	Second ventral dilator of anterior pharynx	37	Tentorium-stomodaeum	13			
18	First ventral dilator of anterior pharynx	36	Tentorium-stomodaeum	14			
19	Clypeal dilator of anterior pharynx	30	Clypeus-stomodaeum	5,6,7,8			
20	?		Superficial muscles of stomodaeum	16			
21	First, second and third frontal dilator of	31,32,	Frons-stomodaeum	11			
22	anterior pharynx	33		10			
23	Occipital dilator of posterior pharynx	38	Vertex-stomodaeum	15			
24	?		-				

CONTINUATION TABLE 2.

	Matsuda, 1965	Graf, 1972		Youssef, 1971	Vilhelmsen, 1996	
1	Posterior fronto-labral			Fronti-labrualis	Posterior labral retractor	3
2	Anterior clypeo-epipharyngeal			Clypeo-epipharyngealis	Retractor of epipharynx	4
3	-			Tentorio-scapualis I	-	
4				Tentorio-scapualis II	-	
5				Tentorio-scapualis III	-	
6				Tentorio-scapualis IV	-	
7	Tergo-mandibular			Cranio-extramandibularis	-	
8	Tergo-mandibular			Cranio-intramandibularis I-II	-	
9	Tergo-cardinal	Protrator cardinal da probóscide	9	Gena-cardinalis	-	
10	Tentorio-cardinal	Adutor da maxila	10	Tentorio-cardinalis	-	
11	Tentorio-stipital	Adutor da maxila	11	Tentorio-stipitalis I	-	
12		Adutor da maxila	12	Tentorio-stipitalis II	-	
13	Postoccipito-premental	Adutor anterior do lábio	1	Postoccipiti-prementualis	Dorsal premental adductor	21
14	Tentorio-premental	Adutor posterior do lábio	2	Tentorio-prementualis	Ventral premental adductors	20
15	Fronto-hypopharyngeal			Fronti-suspensorialis	Retractor of the mouth angle	11
16	Clypeo-hypopharyngeal			Tentorio-suspensorialis	Lateral suspensor of the mouth	12
17	Tantolo-oral			Tentorio-oriscutarius	Ventral cibarial dilatator	8
18	-			Oriscuto-suspensorialis	Ventral pharyngeal dilatator	10
19	Clypeo-dilator of the cibarium			Clypeo-cibarioparietalis	Dorsal cibarial dilator	5
20	Longitudinal muscle of the pharynx			Intersuspensorialis-dorsalis	Longitudinal muscle of the sucking pump / transverse epipharyngeal	6, 7
21	Precerebral dorsal dilator of the pharynx			Fronti-pharyngealis I	Dorsal pharyngeal dilatator	9
22	-			Fronti-pharyngealis II		
23	Posterior dorsal dilator of pharynx			Cranio-pharyngealis	-	
24	Ring muscle			Circulopharyngealis	-	

CONTINUATION TABLE 2.

	Beutel and Vilhelmsen, 2007		Wipfler et al., 2011		Beutel et al., 2014	
1	M. frontoepipharyngalis	9	M. frontoepipharyngalis	0lb2	M. fronto-epipharyngalis	0lb2
2	M. clypeopalatalis	43b	-		-	
3	M. tentorioscapalis posterior	2	M. tentorioscapalis posterior	0an2	M. tentorioscapalis posterior	0an2
4	M. tentorioscapalis lateral	3	M. tentorioscapalis lateral	0an3	M. tentorioscapalis lateral	0an3
5	M. tentorioscapalis median	4	M. tentorioscapalis median	0an4	M. tentorioscapalis median	0an4
6	M. tentorioscapalis anterior	1	M. tentorioscapalis anterior	0an1	M. tentorioscapalis anterior	0an1
7	M. craniomandibularis externus	12	M. craniomandibularis externus	0md2	M. craniomandibularis externus	0md2
8	M. craniomandibularis internus	11	M. craniomandibularis internus	0md1	M. craniomandibularis internus	0md1
9	M. craniocardinalis externus	15	M. craniocardinalis	0mx1	M. craniocardinalis externus	0mx1
10	M. tentoriocardinalis	17	M. tentoriocardinalis	0mx3	M. tentoriocardinalis	0mx3
11	M. tentoriostipitalis	18	M. tentoriostipitalis posterior	0mx5	M. tentoriostipitalis posterior	0mx5
12			M. tentoriostipitalis anterior	0mx4	M. tentoriostipitalis anterior	0mx4
13	-		M. tentorioparaglossalis	0la6	M. tentorioparaglossalis	0la6
14	M. tentoriopraementalis inferior	29	M. tentoriopraementalis	0la5	M. tentoriopraementalis	0la5
15	M. frontohypopharyngalis	41	M. frontooralis	0hy1	M. frontooralis	0hy1
16	-		M. tentoriooralis	0hy2	M. tentoriooralis	0hy2
17	M. tentoriohypopharyngalis	42	M. craniohypopharyngealis	0hy3	M. tentoriohypopharyngalis	0hy3
18	M. longitudinalis stomodaei	69	M. longitudinalis stomodaei	0st2	M. longitudinalis stomodaei	0st2
19	M. clypeopalatalis	43a	M. clypeopalatalis	0ci1	M. clypeobuccalis	0bu1
20	Transverse/longitudinal muscles		M. oralis transversalis	0hy9	M. oralis transversalis	0hy9
21	M. frontobuccalis anterior	45	M. frontobuccalis anterior	0bu2	M. frontobuccalis anterior	0bu2
22	M. frontobuccalis posterior	46	M. frontobuccalis posterior	0bu3	M. frontobuccalis posterior	0bu3
23	M. verticopharyngalis	51	M. verticopharyngalis	0ph1	M. verticopharyngalis	0ph1
24	M. anularis stomodaei	68	M. anularis stomodaei	0st1	M. anularis stomodaei	0st1

CONTINUATION TABLE 2.

	Zimmermann and Vilhelmsen, 2016		HAO 2021	
1	M. fronto-epipharyngalis	0lb2	Posterior fronto-labral muscle	HAO_0000749
2	M. clypeopalatalis	0ci1	Clypeo-epipharyngeal muscle	HAO_0000896
3	M. tentorioscapalis posterior	0an2	Posterior tentorio-scapal muscle	HAO_0001812
4	M. tentorioscapalis lateral	0an3	Lateral tentorio-scapal muscle	HAO_0001777
5	M. tentorioscapalis median	0an4	Median tentorio-scapal muscle	HAO_0001798
6	M. tentorioscapalis anterior	0an1	Anterior tentorio-scapal muscle	HAO_0001164
7	M. craniomandibularis externus	0md2	Posterior cranio-mandibular muscle	HAO_0000108
8	M. craniomandibularis internus	0md1	Anterior cranio-mandibular muscle	HAO_0000745
9	M. craniocardinalis externus	0mx1	Occipito-cardinal muscle	HAO_0001592
10	M. tentoriocardinalis	0mx3	Tentorio-cardinal muscle	HAO_0001638
11	M. tentoriostipitalis posterior	0mx5	Posterior tentorio-stipital muscle	HAO_0002103
12	M. tentoriostipitalis anterior	0mx4	Anterior tentorio-stipital muscle	HAO_0002104
13	-		14	
14	M. tentoriopraementalis	0la5	Anterior tentorio-premental muscle	HAO_0001064
15	M. frontooralis	0hy1	Fronto-sitophoral muscle	HAO_0000897
16	M. tentoriooralis	Ohy2	Tentorio-sitophoral muscle	HAO_0000489
17	M. tentoriohypopharyngalis	0hy3	Tentorio-hypopharyngeal muscle	HAO_0001057
18	M. tentoriopharyngalis	0ph2	Tentorio-pharyngeal muscle	HAO_0001063
19	M. clypeobuccalis	0bu1	19	
20	M. pharyngo-epipharingealis		Pharyngo-epipharyngeal muscle	HAO_0000500
21	M. frontobuccalis anterior	0bu2	Fronto-pharyngeal muscle	HAO_0000262
22	M. frontobuccalis posterior	0bu3		
23	M. verticopharyngalis	0ph1	22	
24	-		23	

TABLE 3. STANDARDIZED TERMINOLOGY FOR THE BEE HEAD MUSCULATURE. HIGHLIGHTED TERMS ARE NEWLY PROPOSED TO THE HAO (2021) CATALOGUE.

Muscle groups	Muscle name		
Labral	Posterior fronto-labral muscle	fro-lab	1
Epipharyngeal	Clypeo-epipharyngeal muscle	cly-epi	2
Antennal	Posterior tentorio-scapal muscle	pten-sca	3
	Lateral tentorio-scapal muscle	lten-sca	4
	Median tentorio-scapal muscle	mten-sca	5
	Anterior tentorio-scapal muscle	aten-sca	6
Mandibular	Anterior cranio-mandibular muscle	acra-man	7
	Posterior cranio-mandibular muscle	pcra-man	8
Maxillar	Geno-cardinal muscle	gen-car	9
	Tentorio-cardinal muscle	ten-car	10
	Anterior tentorio-stipital muscle	aten-sti	11
	Posterior tentorio-stipital muscle	pten-sti	12
Labial	Anterior tentorio-premental muscle	aten-pre	13
	Postoccipito-premental muscle	pos-pre	14
Pharyngeal plate	Fronto-sitophoral muscle	fro-sit	15
	Tentorio-sitophoral muscle	ten-sit	16
	Tentorio-hypopharyngeal muscle	ten-hyp	17
	Tentorio-pharyngeal muscle	ten-pha	18
Anterior cibarial wall	Clypeo-cibarial muscle	cly-cib	19
	Intersitophoral muscle	int-sit	20
Pharyngeal	Anterior fronto-pharyngeal muscle	afro-pha	21
	Posterior fronto-pharyngeal muscle	pfro-pha	22
	Cranio-pharyngeal muscle	cra-pha	23
	Circumpharyngeal muscle	cir-pha	24

5.2.1. LABRAL MUSCLE (Fig. 5)

5.2.1.1. Posterior fronto-labral muscle 1 (HAO_0000749).

Description. A pair of muscles whose fixed attachment point lies on the supraclypeal area, posteriorly to the fronto-sitophoral muscle. From this fixed point, each muscle diverges laterally to reach their movable attachment point on a long tendon at the tormae, which are processes located on the proximolateral end of the labrum. The posterior fronto-labral muscle function as the adductor of the labrum (SNODGRASS, 1942). It seems to correspond to what is called by Snodgrass (1935) the posterior labral muscle of insects.

Variation. Two variations were found for this muscle, the first occurs in Halictinae *sensu stricto* (Augochlorini, Caenohalictini and Halictini species), the fixed attachment point is located more anteriorly in the head, at the supraclypeal area (Fig. 7G), while in *Dufourea* sp. (Rophitini) as well as the other analyzed Apoidea, the fixed attachment point lies on the frontal line, at the supra-antennal area (Fig. 7F, H, I). The second variation refers to the outgroup taxa, *Trypoxylon lactitarse* Saussure, 1867 (Crabronidae) and *Podium* sp. (Sphecidae) and the two cleptoparasites species: *Mesoplia* sp. (Apinae) and *Protosiris mcginleyi* (Apinae), which have the fixed attachment point located anterior to the fronto-sitophoral muscle (Fig. 7I) and not posterior to it, as the others analyzed species (Fig. 7F-H).

5.2.2. EPIPHARYNGEAL MUSCLE (Fig. 5)

5.2.2.1. Clypeo-epipharyngeal muscle 2 (HAO_0000896).

Description. The fixed attachment point is located at the clypeus next to the clypeolabral suture and the movable point is located on the inner wall of the epipharynx. It is a group of divergent fibers whose function is to retract the epipharyngeal wall in *A. mellifera* (SNODGRASS, 1942).

Variation. No significant variation was found.

5.2.3. ANTENNAL MUSCLES (Fig. 4)

No significant variation was found for this group of muscles.

5.2.3.1. Posterior tentorio-scapal muscle 3 (HAO_0001812).

Description. This muscle has its fixed attachment point on the inner margin of the posterior part of the anterior tentorial arm concave surface, close to the tentorium posterior connection, and on the secondary tentorial bridge. The movable attachment point is located medially and dorsally on a tendon like apodemal growth of the base of the antennal scape. This muscle function, together with the lateral tentorio-scapal muscle, as the levators of the antenna, therefore, they lift the scape vertically.

5.2.3.2. Lateral tentorio-scapal muscle 4 (HAO_0001777).

Description. The fixed attachment point of this muscle is located on the outer surface of the posterior part of the anterior tentorial arm, lateral to the anterior and median tentorio-scapal muscles. The movable attachment point is laterally and dorsally on the antennal scape. This muscle function, together with the posterior tentorio-scapal muscle, as the levators of the antenna, therefore, they lift the scape vertically.

5.2.3.3. Median tentorio-scapal muscle 5 (HAO_0001798).

Description. The fixed attachment point of this muscle is located at the inner margin of the anterior tentorial arm, anteriorly to the posterior tentorio-scapal muscle. The movable attachment point is ventrolateral on the antennal scape, in the opposite position of the posterior tentorio-scapal muscle. This muscle function, together with the anterior tentorio-scapal muscle, as the depressors of the antenna, therefore they lower the scape vertically.

5.2.3.4. Anterior tentorio-scapal muscle 6 (HAO_0001164).

Description. This muscle has its fixed attachment point on the anteromedially part of the anterior tentorial arm, right above the lateral and median tentorio-scapal muscles. The movable point is located ventromedially on the antennal scape. This muscle function, together with the median tentorio-scapal muscle, as the depressors of the antenna, therefore they lower the scape vertically.

5.2.4. MANDIBULAR MUSCLES (Fig. 3)

5.2.4.1. Anterior cranio-mandibular muscle 7 (HAO_0000745).

Description. This muscle has two branches, I and II (Fig. 3). The branch I has several bundles of fibers, some of which have their fixed attachment point on the postgena, below the foramen, however, the vast majority is distributed in the genal area behind the compound eye. Branch II has its fixed attachment point on the vertex of the cranium, ventrolaterally to the lateral ocelli. The movable point of this muscle is on a stout apodeme at the adductor angle on the inner side of the mandible. This stout apodeme ramifies into two apodemal growths, the one that gives rise to the branch I is short and robust, and the other one which gives rise to branch II is thin and long. This muscle corresponds to what has been called the “abductor muscle of the mandible” in other groups such as Formicidae (RICHTER, 2019), Vespidae (ALAM, 1951) and Xyelidae (BEUTEL AND VILHELMSEN, 2007, BEUTEL, 2008).

Variation. In females, this muscle tends to have many variations in size but none considered meaningful.

Macrocephalic and non-macrocephalic males did not show significant distinction except for greater robustness of the general mandibular musculature presented by macrocephalic males (Figs. 22, 30) compared to non-macrocephalic males (Figs. 16, 25, 30, 33, 62, 103, 117). Males have more robust bundles than females but still have the general pattern of Apidae and the only variation found is

related to an accessory origin of the branch I in the posterior insertion of the tentorium (Fig. 7B) that is found in Andreninae males only.

5.2.4.2. Posterior cranio-mandibular muscle 8 (HAO_0000108).

Description. The fixed attachment point of this muscle is located at the genal area of the cranium, behind the compound eye and under the branch I of the anterior cranio-mandibular muscle, the movable attachment point is located on the abductor swelling on the outer side of the base of the mandible.

Variation. In all the analyzed species, this muscle tends to vary in size, but no meaningful modification in shape was found. Macrocephalic and non-macrocephalic males did not show significant distinction except for greater robustness of the general mandibular musculature that macrocephalic males have (Figs. 22, 30) compared to non-macrocephalic males (Figs. 16, 25, 30, 33, 62, 103, 117).

5.2.5. MAXILLARY MUSCLES (Fig. 4, 5 and 6)

5.2.5.1. Geno-cardinal muscle 9 (HAO_0001592).

Description. This muscle has its fixed attachment point mainly in the hypostomal region, being almost parallel to the main axis of the head. The movable attachment point is on the small arm located beyond the cranial articulation of the cardo. According to Snodgrass (1942), this muscle is a protractor of the maxilla. This muscle seems to be the correspondent of the occipito-cardinal muscle (HAO_0001592) on HAO (2021) however, here this structure will be referred to as the as geno-cardinal muscle as explained in the sections 5.3 and 6.5.

Variation. It is very similar in most studied species, the fixed attachment point is located posteromedially on the postgena, adjacent to the proboscidial fossa, being positioned perpendicular to the main axis of the head in the median region (Fig. 7B), while in Halictinae species it is positioned almost parallel to the main axis of the head

(Fig. 7C). The fixed attachment point also changes in these species, being mainly in the hypostomal region. *Thygater analis* (Lepeletier, 1841) (Apinae) has this muscle subdivided (Fig. 58), a unique feature among the studied species.

5.2.5.2. Tentorio-cardinal muscle 10 (HAO_0001638).

Description. This muscle in its median extension joins the anterior and posterior tentorio-stipital forming the maxillary complex of proboscidial muscles. The fixed attachment point is located on the anterior end of the anterior tentorial arm and the movable point is at the conjunctiva between the proximal end of the stipes and the distal end of the cardo at the cardino-stipital hinge, a membranous area linking the cardo and the stipe (SELTMAN, 2010 at HAO, 2021).

Variation. In most Apidae species analyzed, in *T. lactitarse* (Crabronidae) and *Podium* sp. (Sphecidae) this muscle is very similar, it is not fused with the other maxillary muscles, and its fixed and movable attachment points are similar to the description of *M. sodalis* (Fig. 7F, H). In Halictinae *s.s*. this muscle has the same pattern as *M. sodalis.* (Fig. 7G).

5.2.5.3. Anterior tentorio-stipital muscle 11 (HAO_0002104).

Description. The anterior tentorio-stipital muscle joins the posterior tentorio-stipital muscle in most of his median extension and additionally joins the tentorio-cardinal muscle forming the maxillary complex of proboscidial muscles. This muscle has its fixed attachment point laterally on the inner side of the anterior tentorial arm, under the combined fixed attachment points of the tentorio-cardinal muscle and the posterior tentorio-stipital muscle. Its movable attachment point is on the inner wall of the base of the stipes.

Variation. In long-tongued bees the pattern of this muscle is similar. The fixed attachment point is located laterally on the inner side of the eutentorial arm, under the combined fixed attachment points of tentorio-cardinal muscle and posterior tentorio-

stipital muscle. Its movable attachment point is on the inner wall of the base of the stipes, right above the one of the posterior tentorio-stipital muscle (Fig. 7H). In Andreninae, Colletinae, Melittinae and wasp species this muscle is fused with the posterior tentorio-stipital muscle, similar to *M. sodalis* (Fig. 7G). In *Dufourea* sp. (Rophitini) something similar occurs, the anterior tentorio-stipital muscle joins the posterior tentorio-stipital muscle (Fig. 114), but the remaining Halictinae species have the same pattern of *M. sodalis* (Fig. 7G).

5.2.5.4. Posterior tentorio-stipital muscle 12 (HAO_0001638).

Description. The posterior tentorio-stipital muscle joins the anterior tentorio-stipital muscle in most of its median extension and additionally joins the tentorio-cardinal muscle forming the maxillary complex of proboscidial muscles. The fixed attachment point of this muscle is on the anterior ventral margin of the anterior tentorial arm, under that of the tentorio-cardinal muscle. The movable point is located medially on the inner wall at the base of the stipes.

Variation. The topology of this muscle is similar among the species analyzed of Apinae and Megachilinae, the fixed attachment point of this muscle is on the anterior ventral margin of the eutentorial arm, under that of the tentorio-cardinal muscle. The movable point is located medially on the inner wall at the base of the stipes, posterior to that of the anterior tentorio-stipital muscle (Fig. 7H). In the remaining analyzed species this muscle is fused with the anterior tentorio-stipital muscle, as already mentioned in the previous section (Fig. 7F, G, I). *Megachile apicipennis* Schrottky, 1902 (Megachilinae) has this muscle ramified close to the fixed attachment point (Fig. 125); despite this, it has the typical topology of this subfamily.

5.2.6. LABIAL MUSCLES (Fig. 5 and 6)

5.2.6.1. Anterior tentorio-premental muscle 13 (HAO_00010640).

Description. The fixed attachment point of this muscle is located on the anterior end of the anterior tentorial arm. From this point, this muscle joins the postoccipito-premental muscle forming the labial branch of the proboscidial muscles and then converges with its pair from the opposite side of the head, and the two are fixed on a single tendonlike apodemal growth located on the proximal end of the prementum. This muscle probably functions as the posterior adductor of the labium (SNODGRASS, 1942).

Variation. In most species analyzed, this muscle is very similar, the fixed and movable attachment points are similar to the ones of *M. sodalis*, except that it is not fused (Fig. 7F, H). In all the Halictinae species the pattern is the same as *M. sodalis* forming the labial branch of the proboscidial muscles (Fig. 7G). One variation was found regarding the movable attachment point, in the Apidae species analyzed, the homologues on the left and right side of the head are inserted in a single tendon at the base of the prementum (Fig. 7F), while in the outgroup species each homologue is inserted at an individual tendon at the base of the prementum (Fig. 7I).

5.2.6.2. Postoccipito-premental muscle 14

Description. This muscle is fused to the anterior tentorio-premental muscle forming the labial branch of the proboscidial muscles, and its fixed attachment point is in the anterior end of the anterior tentorial arm. The movable point of attachment is located on the apodemal growth at the ligular arm of the prementum. The ligular arm is a ligament located on the base of the cluster composed of the glossa and paraglossae. The function of this muscle is to be the anterior adductor of the labium (SNODGRASS, 1942). This muscle is not tied to any concept available in HAO (2021), therefore, the use of the term postoccipito-premental muscle (Matsuda, 1965) is indicated to designate it in this study, as specified in the sections 5.3 and 6.6.

Variation. Three main sources of variation were found for this muscle mainly concerning the fixed attachment point. The species analyzed of Andreninae, Melittinae and part of Colletinae (*Hylaeus* sp. and *Tetraglossula anthracina* (Michener, 1989)) have this muscle with its fixed attachment point in the cavity formed by the fan-shaped sheet of the posterior tentorial arm (Fig. 7B) in the postoccipital region. The exclusive pattern of Halictinae is that described for *M. sodalis* (Fig. 7G). Finally, in the Apinae two patterns appear, in *A. mellifera*, *Xylocopa augusti* Lepeletier, 1841, and in both Meliponini species (*Scaptotrigona bipunctata* (Lepeletier, 1836) and *Melipona quadrifasciata* Lepeletier, 1836) this muscle has its fixed attachment point in the occiput, a pattern that is also found in the Colletinae species *Ptilogossa* sp. and *Colletes petropolitanus* Dalla Torre, 1896 and in the species of Megachilinae analyzed (Figs. 7A), while the remaining Apinae have the pattern that was first described (Fig. 7B). In *Podium* sp. (Sphecidae) this muscle has the pattern in which it has its fixed attachment point in the cavity formed by the fan-shaped sheet of the posterior tentorial arm (Fig. 7B). *T. lactitarse* (Crabronidae) has the pattern where this muscle has its fixed attachment point in the occiput and his movable attachment point is the same as the one of the studied Apidae (Fig. 7A).

5.2.7. MUSCLES OF THE PHARYNGEAL PLATE (Fig. 5)

5.2.7.1. Fronto-sitophoral muscle 15 (HAO_0000897)

Description. The fixed attachment point of this muscle is on the supraclypeal area, anteriorly to the posterior fronto-labral muscle, and below the median ocellus. The movable point is located dorsally on the so-called apodeme for frontal muscles (PORTO AND ALMEIDA, 2019) at the pharyngeal rod, in the opposite position to that of the tentorio-sitophoral muscle. Snodgrass (1942) considered this muscle to be the retractor of the pharyngeal plate.

Variation. three sources of variation for this muscle were found. First, this muscle may have its fixed attachment point at the supraclypeal area (Fig. 7G), as in the Halictinae analyzed species of Augochlorini, Caenohalictini and Halictini or at the supra-antennal

area, which is the case of the remaining species (Fig. F, H, I). The second variation refers to the movable point of attachment, in almost all analyzed species it is precisely in the apodeme for frontal muscles, but in *M. apicipennis* (Megachilinae) and *Dufourea* sp. (Halictinae) the movable point is located broadly along the pharyngeal rod (Figs. 114, 125). And the third variation concerns the outgroup species and the cleptoparasites *Mesoplia* sp. (Apinae) and *Protosiris mcginleyi*. (Apinae). In this species this muscle has its fixed attachment point at the supra-antennal area, as in most analyzed species of the Apidae subfamilies, but it is positioned posteriorly to the posterior fronto-labral muscle (Fig. 7I) and not anteriorly as it is in most of the Apidae species (Fig. 7F-H).

5.2.7.2. Tentorio-sitophoral muscle 16 (HAO_0000489)

Description. The fixed attachment point is on the inner margin of the anterior tentorial arm. The movable one is more easily located on the apodeme for frontal muscles at the pharyngeal rods, in the opposite position of the fronto-sitophoral muscle. This muscle functions as a protractor of the pharyngeal plate.

Variation. No considerable variation was found.

5.2.7.3. Tentorio-hypopharyngeal muscle 17 (HAO_0001057)

Description. This is an unpaired muscle and his fixed attachment point is located on a tendon arising from the median process of the tentorial bridge and the broad movable attachment point is located proximally on the pharyngeal plate at the hypopharyngeal lobe. It functions as a pharynx dilator, when contracted, it pulls the pharyngeal plate dilating the pharyngeal walls, thus opening the mouth (SNODGRASS, 1942).

Variation. No considerable variation was found.

5.2.7.4. Tentorio-pharyngeal muscle 18 (HAO_0001063)

Description. This muscle arises on the dorsal surface of the tentorial bridge, laterally to the tentorio-hypopharyngeal muscle, runs lateroposteriorly on the pharyngeal wall, beneath the circumpharyngeal muscle, and inserts itself on the sitophore and pharyngeal rods at the pharyngeal plate. According to Snodgrass (1942) this muscle functions as a contractor of the pharynx, but judging by the possible contracting movement of this muscle, that is, when contracted it pulls on the pharyngeal rods, it seems more to act as a pharynx dilator.

Variation. No considerable variation was found.

5.2.8. ANTERIOR CIBARIAL WALL MUSCLES (Fig. 5)

5.2.8.1. Clypeo-cibarial muscle 19

Description. It consists of five pairs of muscle bundles and their fixed attachment points are on the internal wall of the clypeus, parallel to the epistomal ridge. From this point, they diverge internally to the anterior cibarial wall inserting themselves between the bundles of the intersitophoral muscle. Snodgrass (1942) considered these muscles to be the dilators of the cibarium. This muscle is not tied to any concept available in HAO (2021), therefore, here this structure will be referred to as the clypeo-cibarial muscle to designate it as specified in the sections 5.3 and 6.8.

Variation. No considerable variation was found in the analyzed bee species, but in *T. lactitarse* (Crabronidae) and *Podium* sp. (Sphecidae) this muscle is not paired and is composed of a central line of bundles (Fig.7E) instead of two parallel lateral lines of bundles (Fig. 7D) as in the analyzed Apidae species.

5.2.8.2. Intersitophoral muscle 20

Description. This muscle consists of many bundles (at least 13) extending between the two pharyngeal rods of the pharyngeal plate. It functions as the compressor/constrictor of the cibarium (SNODGRASS, 1942). This muscle is not tied to any concept available in HAO (2021), therefore, here this structure will be referred to as the intersitophoral muscle to designate it in this study as specified in the sections 5.3 and 6.8.

Variation. No significant variation was found.

5.2.9. PHARYNGEAL MUSCLES (Fig. 5)

5.2.9.1. Anterior fronto-pharyngeal muscle 21

Description. This is a muscle composed of two bundles. Some authors treat them as fronto-pharyngeal I and II, however in this work I'll treat them only as anterior fronto-pharyngeal muscle. The fixed attachment point is located at the supraclypeal area. The movable point is located on the pharynx between the bundles of the intersitophoral muscle.

Variation. Only one variation was found for this muscle. In all the species of Apidae analyzed, this muscle is composed of two bundles (Fig. 7F-H), but in the outgroup taxa only one central continuous muscle bundle was found (Fig. 7I).

5.2.9.2. Posterior fronto-pharyngeal muscle 22

This is a muscle that was found only in the analyzed species of the Meliponini tribe (*M. quadrifasciata* and *S. bipunctata*, Figs. 66, 70).

Description. This is a muscle composed of six small bundles. Its origin is located at the posterodorsal region of the frons, posteriorly to the fixed attachment point of the

frontal muscles. The insertion point is located on the posteriormost part of the pharynx between the bundles of the intersitophoral muscle.

5.2.9.3. Cranio-pharyngeal muscle 23

Description. This muscle has its fixed attachment point on the vertex and the movable point is on the posterior portion of the pharynx right in front of the corpus cardiac. This muscle functions as a dilator of the posterior portion of the pharynx (SNODGRASS, 1942). This muscle is not tied to any concept available in HAO (2021), therefore, here this structure will be referred to as the cranio-pharyngeal muscle to designate it in this study as specified in the sections 5.3 and 6.9.

Variation. No variation was found.

5.2.9.4. Circumpharyngeal muscle 24

Description. This muscle is composed of ring-shaped bundles that cover the pharynx throughout the extension of the pharyngeal rods covering part of the tentorio-pharyngeal muscle. In general, the indicated function of this muscle is of the constrictor of the pharyngeal walls (SNODGRASS, 1942). This muscle is not tied to any concept available in HAO (2021), therefore, here this structure will be referred to as the circumpharyngeal muscle to designate it in this study as specified in the sections 5.3 and 6.9.

Variation. No significant variation was found.

5.3. TERMINOLOGICAL PROPOSALS (Table 3)

Circumpharyngeal muscle 24 (Fig. 5)

Definition

The pharyngeal muscle is composed of ring-shaped bundles that cover the Tentorio-sitophoral muscle and ensheathe the part of the pharynx posterior to the pharyngeal rods for a variable extension.

Synonyms

Intrinsic muscle of the pharyngeal diverticulum (DUNCAN, 1939)

Constrictors of the pharynx (SNODGRASS, 1942)

Ring muscle (MATSUDA, 1965)

Circulopharyngealis (YOUSSEF, 1971)

M. anularis stomodaei (BEUTEL and VILHELMSEN, 2007)

Clypeo-cibarial muscle 19 (Fig. 5)

Definition

A muscle that arises from the clypeus internal wall, parallel to the epistomal sulcus, and inserts on the anterior cibarial wall, between the bundles of the Intersitophoral muscle.

Synonyms

Dilator pharynges (MORISON, 1927)

Dilator buccalis (DUNCAN, 1939)

Dilators of the suctorium (SNODGRASS, 1942)

Clypeal dilator of anterior pharynx (ALAM, 1951)

Clypeus-stomodaeum (MATSUDA, 1957)

Clypeo-dilator of the cibarium (MATSUDA, 1965)

Clypeo-cibarioparietalis (YOUSSEF, 1971)

Dorsal cibarial dilator (VILHELMSEN, 1996)

M. clypeopalatalis (BEUTEL, 2007)

Cranio-pharyngeal muscle 23 (Fig. 5)

Definition

The pharyngeal muscle that arises on the vertex and inserts on the posterior portion of the pharynx, in front of the *corpus cardiacum*.

Synonyms

Dorsal dilator of posterior pharynx (DUNCAN, 1939)

Postecerebral muscle of pharynx (SNODGRASS, 1942)

Occipital dilator of posterior pharynx (ALAM, 1951)

Vertex-stomodaeum (MATSUDA, 1957)

Posterior dorsal dilator of pharynx (MATSUDA, 1965)

Cranio-pharyngealis (YOUSSEF, 1971)

M. verticopharyngalis (BEUTEL AND VILHELMSEN, 2007)

Anterior fronto-pharyngeal 21 (Fig. 5)

Definition

The muscle composed of two bundles that arises in the supraclypeal region and inserts in the pharynx between the bundles of the intersitophoral muscle.

Synonyms

Dilator pharyngis (MORISON, 1927)

Frontal dilator of anterior pharynx (DUNCAN, 1939)

Precerebral dilators of pharynx (SNODGRASS, 1942)

Frons-stomodaeum (MATSUDA, 1957)

Precerebral dorsal dilator of the pharynx (MATSUDA, 1965)

Fronti-pharyngealis I and II (YOUSSEF, 1971)

Dorsal pharyngeal dilatator (VILHELMSEN, 1996)

M. frontobuccalis anterior and posterior (BEUTEL AND VILHELMSEN, 2007)

Posterior fronto-pharyngeal 22 (Fig. 37, 60)

Definition

The muscle composed of six small bundles that arises in the posterodorsal region of the frons, posteriorly to the fixed attachment point of the frontal muscles and

inserts in the posteriormost part of the pharynx between the bundles of the intersitophoral muscle.

Comments

In the analyzed bee species, I only found this muscle in the analyzed species of the Meliponini tribe (*S. bipunctata* and *M. quadrifasciata*, Figs. 37, 60).

Geno-cardinal muscle 9 (Fig. 4)

Definition

The maxillary muscle that arises medially from the gena and/or postgena laterally of the occipital foramen and inserts on the proximolateral part of the cardo.

Synonyms

Productor of the cardo (DUNCAN, 1939)

Cardinal protractor of the proboscis (SNODGRASS, 1942

First protractor of the maxilla (ALAM, 1951)

Gena-cardinalis (Youssef, 1971)

Cardinal protractor of the proboscis (PRENTICE, 1998)

M. craniocardinalis externus (BEUTEL AND VILHELMSEN, 2007)

Comments

This muscle has, in the study group, a topology in which its origin is stable in the genal region, so I believe that the most appropriate term to refer to this structure is geno-cardinal muscle.

Intersitophoral muscle 20 (Fig. 5)

Definition

The muscle that runs anteriorly on the sitophore, between the pharyngeal rods at the anterior cibarial wall.

Synonyms

Pharyngeal compressor (MORISON, 1927)

Pharyngeal dilator of the mouth (DUNCAN, 1939)

Compressors of the suctorium (SNODGRASS, 1942)

Superficial muscles of stomodaeum (MATSUDA, 1957)

Longitudinal muscle of the pharynx (MATSUDA, 1965)

Intersuspensorialis-dorsalis (YOUSSEF, 1971)

Longitudinal muscle of the sucking pump (VILHELMSEN, 1996)

M. longitudinalis stomodaei (BEUTEL AND VILHELMSEN, 2007)

Postoccipito-premental muscle 14 (Figs. 5, 6)

Definition

The muscle that arises on the postoccipital area of the cranium and inserts at the proximal ligament of the ligular arm.

Synonyms

Retractor linguae longus (WOLFF, 1875)

Retractor ligulae superior (MORISON, 1927)

Flexor of paraglossae (DUNCAN, 1939)

Anterior adductor of the labium (SNODGRASS, 1942)

Posterior adductor **(**ALAM, 1951)

Tentorium-prementum (MATSUDA, 1957)

Postoccipito-premental (MATSUDA, 1965)

Postoccipiti-prementualis (YOUSSEF, 1971)

Dorsal premental adductor (VILHELMSEN, 1996)

Comments

The suggested name for this muscle is postoccipito-premental of Matsuda (1965).

In Apidae this muscle appears to be much variable with at least three distinct points of origin: the occipital area, in the insertion of the posterior tentorial arm (postoccipital area), and in the insertion of the anterior tentorial arm.

5.4. CHARACTER STATEMENTS

Below are described the 12 morphological characters and their respective states derived from the extrinsic head musculature of the Apidae, which were scored for 34 bee species plus the outgroup (Table 4). These characters summarize the overall morphological variation described in the previous sections and are interpreted in a phylogenetic context being optimized onto a phylogenetic hypothesis. The only

strong sexual dimorphism was related to one mandibular muscle and is described in section 5.2.4.1. and discussed in section 6.4.

5.4.1. Female based characters

01 – Posterior fronto-labral muscle, front, origin: (0) at the supra-antennal area (Fig. 7F, H, I); (1) at the supraclypeal area (Fig. 7G).
02 – Posterior fronto-labral muscle, front, position relative to the fronto-sitophoral muscle: (0) posterior (Fig. 7F-H); (1) anterior (Fig. 7I).

03 – Geno-cardinal muscle, gena, position relative to the main axis of the head: (0) perpendicular (Fig. 7B); (1) parallel (Fig. 7C).

04 - Maxillary complex of proboscidial muscles, tentorium: (0) absent (Fig. 7F, H, I); (1) present (Fig. 7G).

05 – Anterior and posterior tentorio-stipital muscles, tentorium: (0) separated (Fig. 7H); (1) fused (Fig. 7F, G, I).

06 - Labial complex of proboscidial muscles, tentorium: (0) absent (Fig. 7F, H, I); (1) present (Fig. 7G).

07 - Anterior tentorio-premental muscle, tentorium, insertion: (0) attaching on a unique tendon at the base of the prementum (Fig. 7F); (1) attaching on individual tendons at the base of the prementum (Fig. 7I).

08 - Postoccipito-premental muscle, postocciput, origin: (0) in the occiput (Fig. 7A); (1) in the cavity at the insertion of posterior tentorial arm (postoccipital area) (Fig. 7B); (2) fused in the labial complex of the proboscidial muscles (Fig. 7G).

09 - Clypeo-cibarial muscle, clypeo: (0) paired and lateralized (Fig. 7D); (1) single and centralized (Fig. 7E).

10 – Anterior fronto-pharyngeal muscle, front: (0) two bundles (Fig. 7F-H); (1) one single bundle (Fig. 7I).
11 – Posterior fronto-pharyngeal muscle, front: (0) present (Fig. 7H); (1) absent (Fig. 7F, G, I).

5.4.2. Male based characters

12 – Anterior cranio-mandibular muscle, gena, origin: (0) only in the genal area (Fig. 7A); (1) in the genal area and in the insertion of the posterior tentorial arm (postoccipital area) (Fig. 7B).

TABLE 4. CHARACTER MATRIX FOR THE BEE HEAD MUSCULATURE.

SPECIES	01	02	03	04	05	06	07	08	09	10	11	12
Podium sp.	0	1	0	0	1	0	1	1	1	1	1	?
Trypoxylon lactitarse	0	1	0	0	1	0	1	0	1	1	1	?
Andrena sp.	0	0	0	0	1	0	0	1	0	0	1	?
Callonychium petuniae	0	0	0	0	1	0	0	1	0	0	1	1
Psaenythia annulata	0	0	0	0	1	0	0	1	0	0	1	1
Psaenythia bergii	0	0	0	0	1	0	0	1	0	0	1	1
Anthrenoides meridionalis	0	0	0	0	1	0	0	1	0	0	1	1
Rhophitulus sp.	0	0	0	0	1	0	0	1	0	0	1	?
Apis mellifera	0	0	0	0	0	0	0	0	0	0	1	?
Bombus morio	0	0	0	0	0	0	0	1	0	0	1	?
Centris tarsata	0	0	0	0	0	0	0	1	0	0	1	?
Melitoma segmentaria	0	0	0	0	0	0	0	1	0	0	1	?
Mesoplia sp.	0	1	0	0	0	0	0	1	0	0	1	?
Melissoptila aureocincta	0	0	0	0	0	0	0	1	0	0	1	?
Thygater analis	0	0	0	0	0	0	0	1	0	0	1	?
Eulaema cingulata	0	0	0	0	0	0	0	1	0	0	1	0
Melipona quadrifasciata	0	0	0	0	0	0	0	0	0	0	0	?
Scaptotrigona bipunctata	0	0	0	0	0	0	0	0	0	0	0	?
Protosiris mcginleyi	0	1	0	0	0	0	0	1	0	0	1	?
Arhysoceble sp.	0	0	0	0	0	0	0	1	0	0	1	?
Tetrapedia diversipes	0	0	0	0	0	0	0	1	0	0	1	?
Ceratina stilbonota	0	0	0	0	0	0	0	1	0	0	1	?
Xylocopa augusti	0	0	0	0	0	0	0	0	0	0	1	?
Colletes petropolitanus	0	0	0	0	1	0	0	0	0	0	1	?
Ptiloglossa sp.	0	0	0	0	1	0	0	0	0	0	1	?
Hylaeus sp.	0	0	0	0	1	0	0	1	0	0	1	?
Tetraglossula anthracina	0	0	0	0	1	0	0	1	0	0	1	?
Dufourea sp.	0	0	1	0	1	1	0	2	0	0	1	?
Augochlora daphnis	1	0	1	1	1	1	0	2	0	0	1	0
Megalopta sodalis	1	0	1	1	1	1	0	2	0	0	1	?
Agapostemon semimelleus	1	0	1	1	1	1	0	2	0	0	1	?
Dialictus opacus	1	0	1	1	1	1	0	2	0	0	1	?
Moureanthidium paranaense	0	0	0	0	0	0	0	0	0	0	1	0
Coelioxys sp.	0	0	0	0	0	0	0	0	0	0	1	?
Megachile apicipennis	0	0	0	0	0	0	0	0	0	0	1	?
Melitta tricincta	0	0	0	0	1	0	0	1	0	0	1	?

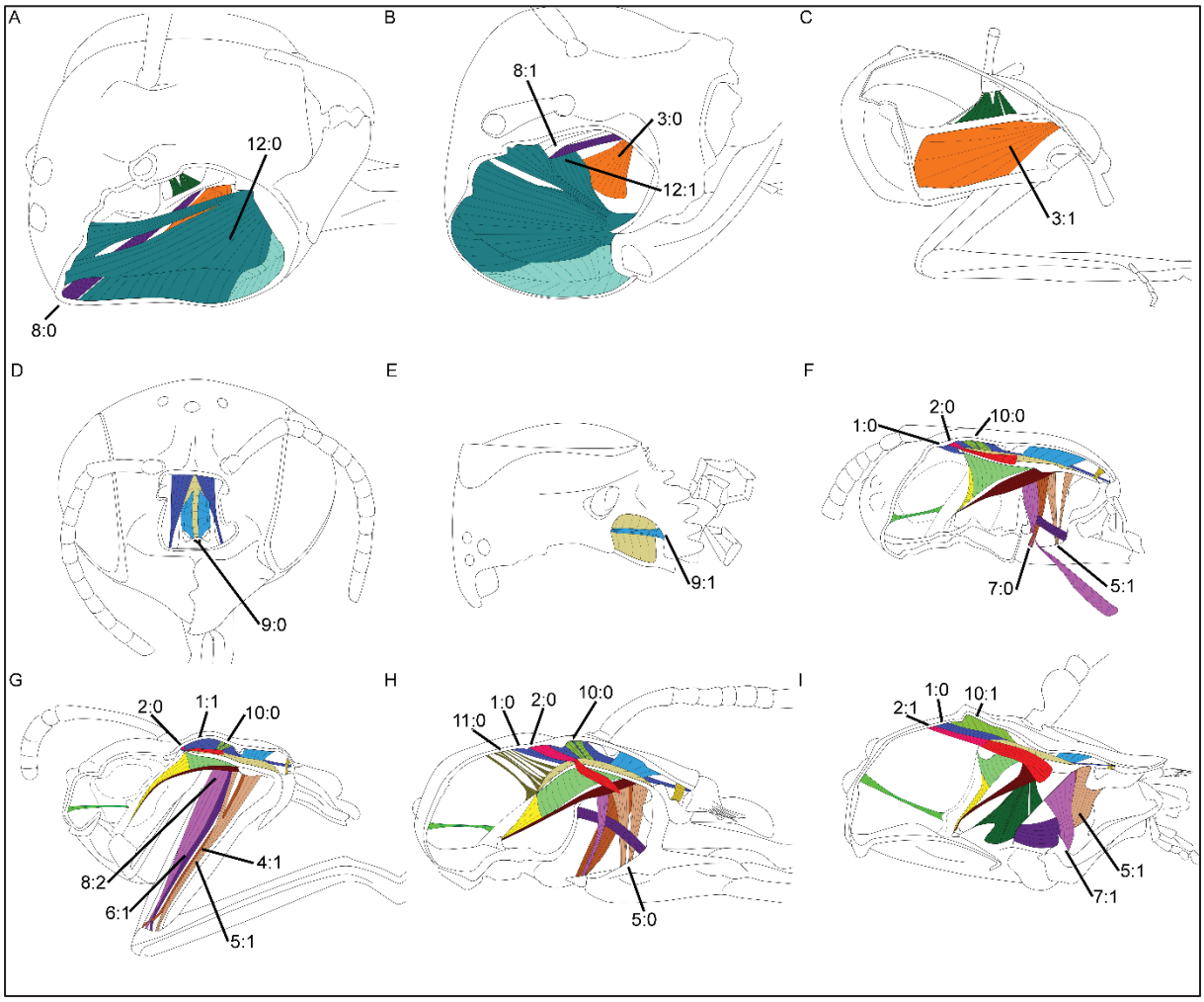

FIGURE 7. COMPARATIVE MORPHOLOGY OF THE BEE HEAD MUSCULATURE. A, LATERAL VIEW OF THE HEAD OF MALE *M. PARANAENSE*; B, LATERAL VIEW OF THE HEAD OF MACROCEPHALIC MALE *P. ANNULATA*; C, LATERAL VIEW OF THE HEAD OF FEMALE *A. DAPHNIS*; D, FRONTAL VIEW OF THE CLYPEO-LABRAL AREA OF FEMALE *M. APICIPENNIS*; E, FRONTAL VIEW OF THE CLYPEO-LABRAL AREA OF FEMALE *PODIUM* SP.; F, LATERAL VIEW OF THE HEAD OF FEMALE *HYLAEUS* SP.; G, LATERAL VIEW OF THE HEAD OF FEMALE *A. SEMIMELLEUS*; H, LATERAL VIEW OF THE HEAD OF FEMALE *S. BIPUNCTATA*; I, LATERAL VIEW OF THE HEAD OF FEMALE *T. LACTITARSE*. NOT TO SCALE.

6 DISCUSSION

6.1. LABRAL MUSCLE

The labrum in most hexapod lineages has one extrinsic muscle, the M. labroepipharyngealis (here the posterior fronto-labral muscle), and additionally Ephemeroptera and also members of Polyneoptera, can have another intrinsic muscle (BEUTEL et al., 2014). According to Snodgrass (1935) and Beutel et al., (2014) in the groudplan of hexapods, the labrum is movable usually by two pairs, one anterior and one posterior, of long extrinsic muscles taking their origins on the upper part of frons and inserting at the labrum, however, one or in some cases, both of them can be reducer or absent. In the studied group, there is only one pair of extrinsic muscles, the posterior fronto-labral muscle.

The most notable variation in bees refers to the positioning of this muscle in the Augochlorini, Caenohalictini and Halictini species (Character 1 (1), Fig. 8). This variation occurs in conjunction with the fronto-sitophoral muscle and reflects a tendency to concentrate most of the frontal musculature in the supraclypeal region in these species. This variation was already noted by Urban (1963; Fig. 2). This conformation of the frontal musculature may be related to the distinct labiomaxillary tube of the clade in a remarkable rearrangement of the anterior head structures.

The fixed attachment point anteriorly to the fronto-sitophoral muscle is found in the outgroup (character 2 (1), Fig. 8) and also in the cleptoparasites *Mesoplia* sp. and *P. mcginleyi*. Zimmermann and Vilhelmsen (2016) found the same state studying *Pison* sp. (Crabronidae) where the 0lb2 muscle (corresponds to the posterior fronto-labral muscle in this work) originates anteriorly from the 0hy1 muscle (corresponds to the fronto-sitophoral muscle in this work). The presence of this muscle is variable among Apocrita, but the presence can be considered a synapomorphy of Apoidea (character 21 (1) on Zimmermann and Vilhelmsen 2016: Fig. 9). Species of cleptoparasite bees tend to have morphological changes related to their lifestyle, usually reduction or absence of structures related to foraging and nesting (MICHENER, 2007). Such bees can have unique internal morphologies, for example Meira and Gonçalves (2018) analyzing the internal skeletal morphology of the cleptoparasite *Temnosoma* sp. found

difficulties in its positioning given the large amount of autapomorphies characteristic of this genus.

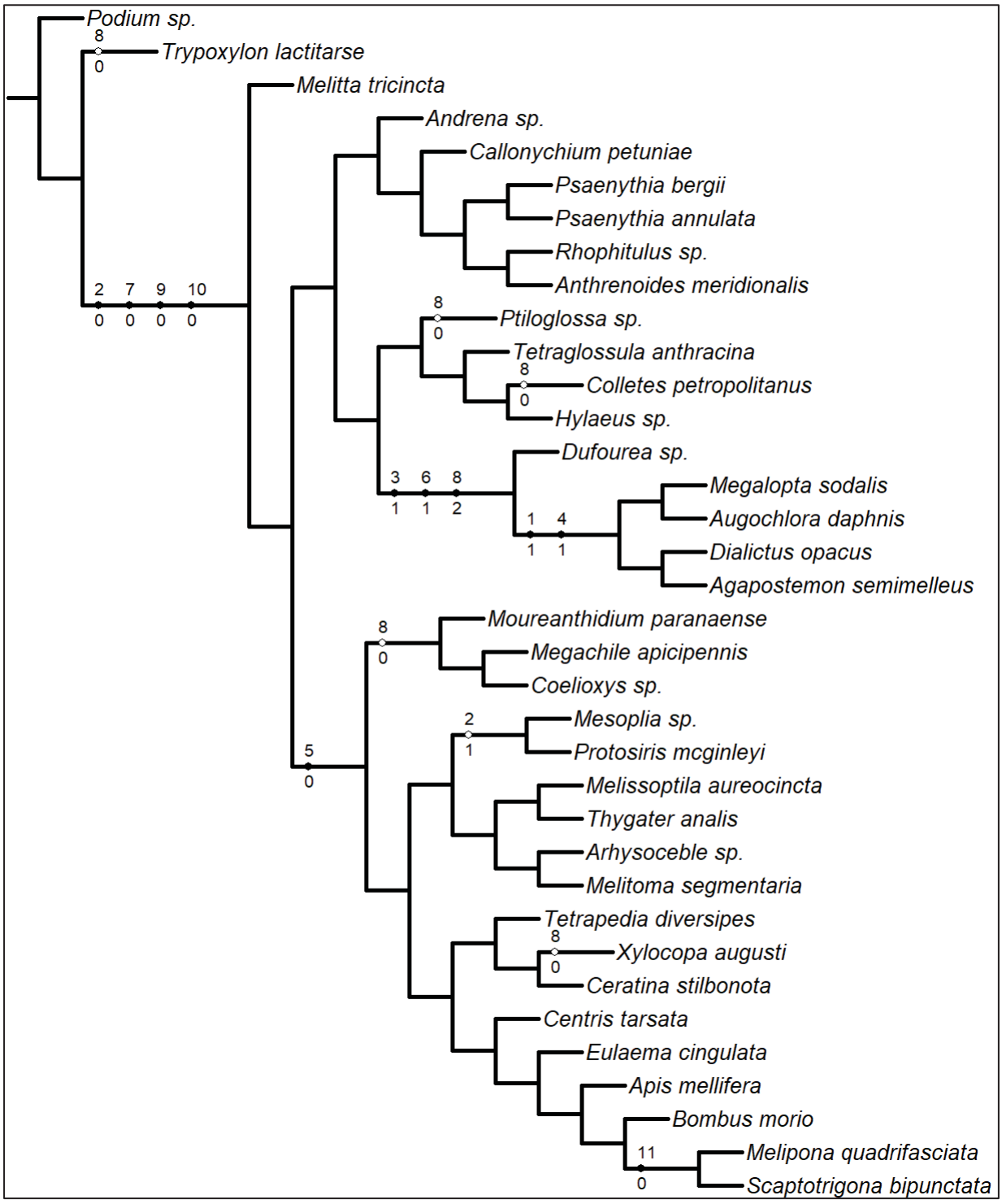

FIGURE 8. UNAMBIGUOUS OPTIMIZATION OF BEE HEAD MUSCULATURE CHARACTERS ON A SUMMARY TREE DERIVED FROM HEDTKE ET AL. (2013). THE CHARACTER STATES (BELOW) AND NUMBERS (ABOVE) REFER TO THE CHARACTER DESCRIPTIONS AT THE CHARACTER LIST 5.4. BLACK DOTS INDICATE NONHOMOPLASIOUS CHARACTERS AND WHITE REFER TO HOMOPLASIOUS CHARACTERS.

6.2. EPIPHARYNGEAL MUSCLE

The epipharyngeal lobe or epipharynx is a type of lobe composed mainly of the conjunctiva, located in front of the oral foramen, at the labrum ventral surface, detailed illustrations can be found in Wolff (1875). In insects, the epipharynx can possess as many as two muscles, one anterior and one posterior (YOUSSEF, 1971). *A. mellifera* has only one epipharyngeal muscle and is probably derived from one of the cibarial dilatators (SNODGRASS, 1942). It is not present in lineages near the base of Hymenoptera (VILHELMSEN, 1996). There was not much variation concerning this muscle and its function should be conserved throughout Apoidea.

6.3. ANTENNAL MUSCLES

The insect antenna is a remarkable sensorial organ composed of three principal parts. The first by which the whole antenna is attached to the head is the scape, the second part articulates with the previous one and is termed the pedicel, and the last one which is made of many small articles represents the flagellum (MICHENER, 2007). The antenna is movable virtually in all directions due to the functions of four extrinsic muscles arising on the dorsal concave surface of the anterior tentorial arm and which are inserted into the base of the scape (SNODGRASS, 1935).

The number and topology of the extrinsic antennal musculature are extremely consistent within Apoidea. This consistency possibly is related to their points of attachment which are located in regions of little modification and by the movement performed by these muscles. Variations are likely to be found at other taxonomic levels and possibly this variation must be directly related to the tentorium shape. Investigations in species with notably distinct antennae, the long-horned bees such as *Trichocerapis mirabilis* (Smith, 1865) (Eucerini), may reveal interesting variations of extrinsic musculature.

6.4. MANDIBULAR MUSCLES

The nonpterygote insects have four mandibular muscles of two types, dorsal and ventral mandibular muscles, however the last type is degenerated in Neoptera insects because of the increasing size of the dorsal adductors, but persist in Lepismatidae, larvae of Ephemeridae and Odonata and in most adult Isoptera and Orthoptera except Acrididae (SNODGRASS, 1935). In Apoidea and other hymenopteran groups such as Xyelidae (BEUTEL AND VILHELMSEN, 2007), Formicidae (RICHTER et al., 2019) and Vespidae (DUNCAN, 1939), each mandible contains two muscles in most species, they are the posterior cranio-mandibular muscle and the anterior cranio-mandibular muscle, abductor, and adductor respectively. This group of muscles is very conserved in Hymenoptera, and the main modifications tend to be related to the fixed attachment points.

The anterior cranio-mandibular muscle is a muscle divided in two branches and, as already noted by Beutel (2008), this is a possible autapomorphy of the Hymenoptera. This muscle seems to be the homologue of the adductor muscle of the mandible (BEUTEL AND VILHELMSEN, 2007; BEUTEL, 2008; NEUGART et al., 2009). The designation anterior, instead of internal, fits better for the study group as this muscle usually lies above and, therefore, anteriorly orientated in relation to the posterior cranio-mandibular muscle. The anterior cranio-mandibular muscle is larger than the posterior one possibly because almost all the work of the mandible falls on it.

Females show no significant variation for this muscle and its function, which is the adduction of the mandibles or the approximation of the opposite mandibles it is a mostly invariable type of movement in dicondylic insects and this implies little structural and topological modification. It is important to note that the mandibular muscles have ramifications, regardless of the subfamilies, indicating that this characteristic would be more linked to subtle differences at lower taxonomic levels that will be better understood if more individuals are added.

The exceptional enlargement in size of the head capsule is a recurrent phenomenon in several groups of Hymenoptera, including bees, wasps and ants (HAMILTON, 1979). It can be expressed in any sex, often as part of a polymorphism or dimorphism (HOUSTON, 1970; HAMILTON, 1979; KUKUK AND SCHWARZ, 1987,

1988; DANFORTH, 1991; MICHENER, 2007). In males, it is usually associated with competition for access to females. Macrocephalic males unable to fly that fight to the death for possession of communal nests have been reported and widely studied in several members of Andreninae and Halictinae (KUKUK AND SCHWARZ, 1987, 1988; DANFORTH, 1991). The studied males have more robust bundles than females, but they generally have the same morphological pattern. The only variation found was related to an accessory origin of the branch I in the posterior insertion of the tentorium (character 12 (1), Fig. 49-50) present only in the studied males of Andreninae, a putative synapomorphy for this subfamily (males of *Andrena* sp. were not studied).

The posterior cranio-mandibular muscle seems to be more conserved among insect groups and appears to be the homologue of the abductor muscle of the mandible in other groups such as Diptera (NEUGART et al., 2009), Formicidae (RICHTER, 2019), Vespidae (ALAM, 1951), Xyelidae (BEUTEL AND VILHELMSEN, 2007; BEUTEL, 2008) and other groups. The designation of this muscle as external or posterior is controversial as both conditions can be found throughout the Apidae, however, the most common disposition of this muscle is posteriorly related to the anterior cranio-mandibular muscle, and therefore this designation seems more appropriate for bees. In this study little variation was found concerning this muscle, probably due to its function, the abduction of the mandible, a movement that shows little modification in bees.

6.5. MAXILLARY MUSCLES

In bees, the maxilla has been modified in many ways (MICHENER, 2007), but the main differences discovered in this study for the Apidae are related to species of some tribes of Halictinae. In this subfamily, there is a change in the anterior region of the proboscis, formed by the union of the anterior and posterior conjunctive thickening and the pair of cardines. This region is slender and the musculature ends up merging making its identification difficult, differently from what happens in the other subfamilies. As the function performed by the extrinsic maxillary musculature is ultimately to protrude the proboscis, the geno-cardinal, tentorio-cardinal and anterior and posterior

tentorio-stipital muscles are collectively known as the protractors of the proboscis. (SNODGRASS, 1942).

The geno-cardinal muscle appears to correspond to the occipito-cardinal muscle in the HAO (2021), however, as in the Apidae this muscle is mainly fixed in the genal area instead of the occipital region, the term geno-cardinal muscle is suggested to designate this muscle as specified in section 5.3. This muscle is remarkably similar in the studied subfamilies except for Halictinae. This occurs because the point of articulation of the cardo with the maxillary process of the hypostoma is displaced anteriorly, being close to the clypeus, this conformation ends up causing this muscle to be arranged almost parallel to the main axis of the head. The variation of the maxillary process of hypostoma was already noted by Michener (1944), Graf (1972), Eickwort (1969), Alexander and Michener (1995; character 22, state 2), Michener (2007), and by Porto et al. (2016b; character 22, state 1). The original fixed point of attachment of this muscle, according to the literature (SNODGRASS, 1942; YOUSSEF, 1971; MIKÓ et al., 2007), is located on the occiput region of the head capsule and the general topology of Apidae is, therefore, a secondary modification. The outgroup species has the same topology as most analyzed species of Apidae subfamilies, the unique topology of Halictinae seems to be a putative synapomorphy of the subfamily (Character 3 (1), Fig. 8). The subdivision that *T. analis* has was not shared by any other species analyzed here.

Halictinae has also a peculiar morphology on the most proximal region of the proboscis manly because of elongation of the basal parts of the proboscis, not only represented by the cardo and anterior and posterior conjunctival thickness but also by the elongation of the prementum (ALEXANDER AND MICHENER, 1995; MICHENER, 2007; EICKWORT, 1969). This peculiar morphology of the labiomaxillary tube found in Halictinae *s.s*. generates a change in the disposition of some maxillary muscles when compared to Rophitini and the remaining bees. In most bees the tentorio-cardinal muscle and the posterior and anterior tentorio-stipital muscles are separately arranged in different forms but in Halictinae s.s. they are united in the maxillary complex of proboscidial muscles (Character 4 (1), Fig. 8). This fusion is comprehensible due to the thinning of the labiomaxillary tube that should be accompanied by an approximation

or even fusion, which is the case for these three muscles, of the musculature analyzed there. Nonetheless, this fusion does not seem to cause many functional changes.

The posterior and anterior tentorio-stipital muscles are treated together by Snodgrass (1942) as the adductors of the maxilla, numbers 12 and 13 in his illustrations. Youssef separated them and apparently made some mistakes in his illustrations since they are unmentioned in his figures and are erroneously nominated in his Figure 4 (YOUSSEF, 1971) as musculus stip-maxipalp, I and II, as the illustration shows that these muscles are connected to the tentorium and the stipes and not to the stipes and the maxillary palpus as the name in the figure suggests. The posterior and anterior tentorio-stipital muscles are remarkably similar in the long-tongued bees (Character 5 (0), Fig. 8) where they are separated, different from all other analyzed species of short-tonged bees plus the outgroup. In the tribes Augochlorini, Caenohalictini and Halictini, the fusion of anterior and posterior tentorio-stipital muscles is accompanied by another fusion with the tentorio-cardinal muscle forming the maxillary complex of proboscidial muscles (Character 4 (1), Fig. 8). The characteristic variation of long-tongued bees (Character 5 (0), Fig. 8) was observed in *Apis mellifera* (SNODGRASS, 1942; YOUSSEF, 1971; GRAF, 1972) and also in *Bombus* sp., but not in *Ceratina assuncionis* Strand, 1910*, Eulaema* sp. and *Megachile laeta* Smith, 1853 (GRAF, 1972). Zimmerman and Vilhelmsen (2016) found the same pattern in *Pison* sp. (Crabronidae), however, in this work the pattern observed in *T. lactitarse* (Crabronidae) was that of short-tongued bees (Character 5 (1), Fig. 8). The characteristic variation of short-tongued bees and the outgroup taxa (Character 5, state 1, Fig. 8) was observed also in *Augochoropsis* sp., *Colletes furfuraceus* Holmberg, 1886 and *Oxaea austera* Gerstaecker, 1867 (GRAF, 1972).

The ramification of *M. apicipennis* at the posterior tentorio-stipital muscle seems to represent a variation at a lower phylogenetic level once it is not shared by the other studied species of the subfamily.

6.6. LABIAL MUSCLES

The labium is the most specialized part of the labiomaxillary tube, therefore exhibiting a more pronounced tendency to change muscle attachment (YOUSSEF,

1971). The only two extrinsic muscles in the labial part of the labiomaxillary tube are the anterior tentorio-premental muscle and the postoccipito-premental muscle, the last one is, in fact, partly intrinsic, in its premental part, and partially extrinsic.

The anterior tentorio-premental muscle appears to be extremely conserved in his morphology in all the subfamilies except for the Halictinae *s. l.*, where similar to what happens with the maxillary muscles, this muscle joins the postoccipito-premental muscle at the labial complex of proboscidial muscles in all evaluated species (Character 6 (1), Fig. 8) possibly this is a synapomorphy of this clade. The characteristic topology of this muscle in the Apidae (Character 7 (0), Fig. 8), where the homologues of each side of the head attach on a single tendon at the base of the prementum, was noted by other authors dealing with *A. mellifera* (SNODGRASS, 1942; YOUSSEF, 1971), *Bombus* sp. and *Augochloropsis* sp. (GRAF, 1972), *Xylocopa* sp. (SNODGRASS, 1935; GRAF, 1965) and several lineages of Apidae (GRAF, 1972). As this seems to be an exclusive feature of the bees and the outgroup has another conformation (Character 7 (1), Fig. 8) this is another possible synapomorphy for the Apidae.

The postoccipito-premental muscle is not listed in HAO catalog (2021), therefore, the use of this term is suggested to designate it as specified in section 5.3. This muscle is the longest one in the bee head and is the one with the most variable topology in this analysis. The characteristic that Halictinae species have (Character 8 (2), Fig. 8) seems to be an unfolding of the specific conformation of the region between the tentorium and the hypostoma. In this taxon, in addition to an anteriorization of the maxillary process of the hypostoma, the tentorium and the hypostoma are fused for almost the entire extent, as commented earlier. In the other bee subfamilies, this muscle originates either in the dorsal arm of the tentorium or in the occiput, passes through the opening between the tentorium and the hypostoma, and then inserts itself in the ligular arm. In Halictinae this passage between the hypostoma and the tentorium is not possible due to the referred conformation, thus, this muscle seems to originate in the anterior insertion of the tentorial arm next to the anterior tentorio-premental muscle at the labial complex of proboscidial muscles. According to Snodgrass (1935), the topology of this muscle in the bees (one of the adductors takes his origins on cranial areas) is a secondary state resulting from a migration of the muscle fibers to increase

effectiveness, and the ancestral condition is the one where this muscle has his fixed attachment point on the tentorium. As emphasized in the previous section to describe the variation of the postoccipito-premental muscle the analyzed species of Andreninae, Melittinae, Colletinae, and part of Apinae have the ancestral condition (Character 8 (1), Fig. 8) where this muscle has its fixed attachment point on the tentorium, while the remaining species of Apinae and Megachilinae have the derived condition (Character 8 (0), Fig. 8). Urban (1963) found similar variations in this muscle where its origin is either in the occiput or the postocciput (possibly in the region of the insertion of the posterior tentorial arm. Graf (1972) found a high degree of variation with respect to the origin of this muscle but the two patterns described here, where this muscle originates either in the occiput or in the posterior insertion of the tentorium, are also described in his study, the variation in Halictinae was not identified.

6.7. MUSCLES OF THE PHARYNGEAL PLATE

Youssef (1971) employed the name Hypopharyngeal Muscles, but in this study the name Muscles of the Pharyngeal Plate is used to designate the following set of muscles that are attached to the hypopharyngeal lobe but also to the other structures that compose the pharyngeal plate. The pharyngeal plate is a complex structure composed of four main components: sitophore, hypopharyngeal lobe, pharyngeal rods, and sometimes a median oral plate (PORTO AND ALMEIDA, 2019).

In Youssef's (1971) study, the name musculus tentorio-suspensorialis was used two times with distinct definitions. It seems that the true musculus tentorio-suspensorialis of his work is the one that is first described, however, here the name tentorio-sitophoral is suggested for this muscle once it inserts itself on the pharyngeal rod which is a support structure of the sitophore (PORTO AND ALMEIDA, 2019). The definition of the second "musculus tentorio-suspensorialis" in Youssef's study (1971) matches with the position indicated to the musculus oriscuto-suspensorialis in Figure 3 (YOUSSEF, 1971), therefore, it is possible that the names were interchanged, however, in this study it is suggested to use the correspondent indicated at HAO (2021) which is "tentorio-pharyngeal muscle" for the muscle that arises at the dorsal surface of the tentorial bridge runs beneath the circumpharyngeal muscle and insert itself on

the sitophore. Lastly, it seems that the muscle oriscuto-suspensorialis described by Youssef (1971) may not exist, since it was not adequately indicated in his illustrations and also not mentioned in further studies. It is suggested the adoption of the term fronto-sitophoral for the muscle that originates in the front and is inserted in the apodeme of the frontal muscles. So, there are four pharyngeal plate muscles: fronto-sitophoral, tentorio-sitophoral, tentorio-hypopharyngeal, and tentorio-pharyngeal.

Concerning the fronto-sitophoral muscle, the variations related to the fixed attachment point are somehow related to the labral musculature and were treated more deeply in this specific topic (6.1). Another difference found here is the movable point of attachment of this muscle, in *M. apicipennis* (Megachilinae) and *Dufourea* sp. (Halictinae) the muscle broadly attaches along the pharyngeal rod and not only in the apodeme for frontal muscles itself, which ends up being restricted to the tentorio-sitophoral muscle.

6.8. ANTERIOR CIBARIAL WALL MUSCLES

The cibarium is the anteriormost part of the alimentary channel and it is delimited proximally by the proximedian part of the pharyngeal plate, anteriorly by the inner clypeal wall, and distally by the functional mouth (VILHELMSEN, 2010 at HAO, 2021). In the sucking insects the cibarium undergoes a remarkable modification and converts itself into the sucking pump by an extension and closure of the lateral true mouth aperture (SNODGRASS, 1935). In bees, the sucking pump is a cibarial/pharyngeal structure (SNODGRASS, 1942) and the muscles of the cibarial region of the sucking pump are the clypeo-cibarial and the intersitophoral muscles.

The clypeo-cibarial muscle is unlinked to any concept at HAO (2021) so here it is suggested to use this name to designate it as specified in section 5.3. Some authors disagree regarding the general number of bundles of this muscle found in *A. mellifera*, for Snodgrass (1942) there are five pairs, whereas for Youssef (1971) the usual number is six pairs, however, it can vary between five and seven bundles. This divergence is possibly due to the high degree of difficulty in observing this muscle since it is small and fragile and, in general, is positioned very close to the clypeal wall, making its dissection and consequent examination difficult. In the studied species a consistent

number of five bundles was found. The characteristic muscle pattern of bees, with the muscle disposed of two lateral series (Character 9 (0), Fig. 8) was already noted by Youssef (1971) in his elegant Figure 1, and the muscular pattern of the outgroup taxa (Character 9 (1), Fig. 8) was already been described by Zimmermann and Vilhelmsen (2016) for *Pison* sp. (Crabronidae). This is a consistent arrangement among bees and it's a putative synapomorphy.

The intersitophoral muscle is not tied to any concept at HAO (2021) so here it is suggested to use this name to designate it as specified in section 5.3. This muscle is probably the most complicated muscle to observe in the bee head. There is some divergence between different authors concerning the exact number of muscle bundles in *A. mellifera*, Snodgrass (1942) described five pairs while Youssef (1971) reported at least 13. The indicated conflict apparently arises from the elevated difficulty to observe both fixed and movable points of attachment to this muscle. In the present study, the same 13 bundles described by Youssef (1971) were found, and this muscle pattern is very consistent in the analyzed species.

6.9. PHARYNGEAL MUSCLES

Matsuda (1965) categorized all the muscles attached to the pharynx, pharyngeal plate, and cibarium as pharyngeal muscles, however, in this study, following Youssef (1971), only the muscles attached to the true pharynx, i.e., the cephalic part of the stomodeum will be classified as pharyngeal muscles. A similar approach was adopted by Snodgrass (1942) classifying this conjunct of muscles as the pharyngeal muscles of the sucking pump. According to Youssef (1971), they can be classified under two subgroups: dorsal (anterior and posterior fronto-pharyngeal and cranio-pharyngeal muscles) and circular (circumpharyngeal muscle).

The anterior fronto-pharyngeal muscles have been found on all groups of insects studied (YOUSSEF, 1971) and they are extremely consistent in the Apidae, and most authors (SNODGRASS, 1935, 1942; YOUSSEF, 1971) agree that they act as a dilator of the anterior part of the pharynx. Bees have a well-established topology for this muscle, is it composed of two bundles (Character 10 (0), Fig. 8) whereas in the

outgroup this muscle is unique and elongated (Character 10 (1), Fig. 8), so this is another possible synapomorphy for the Apidae.

Only the Meliponini species (*S. bipunctata* and *M. quadrifasciata*) possess the posterior fronto-pharyngeal muscle. It is located at the posterodorsal region of the pharynx, posteriorly to the fixed attachment point of the frontal muscles (Character 11 (0), Fig. 8). In *M. quadrifasciata* (Fig. 66) the muscle fibers are more approximated making it look like a single muscle, however, in this study at least six individual small bundles were counted, but in *S. bipunctata* (Fig. 70) the fibers are more separated and it is clear that there are six individual minute bundles. It appears that this muscle is related to the length of the posterior part of the pharyngeal rod in the Meliponini species. Porto and Almeida (2019) noted that bees exhibit in general long pharyngeal rods in comparison to other Apoidea. In this study the Meliponini species exhibit the longest pharyngeal rods at all and, as this structure grows longer, it possible demands more muscle fibers to function as additional dilators of the pharynx.

The cranio-pharyngeal muscle is not tied to any concept at HAO (2021), so here it is suggested to use this name to designate it as specified in section 5.3. This muscle is the second dorsal pharynx dilator and, in this analysis, it does not show considerable variation, being present in all studied species.

The circumpharyngeal muscle is also described as ring muscle and it seems to be very common among Hymenoptera as depicted for Formicidae (RICHTER, 2019), Vespidae (ALAM, 1951), and Xyelidae (BEUTEL AND VILHELMSEN, 2007, BEUTEL, 2008), and it is also common in other insects such as Lepidoptera (Snodgrass, 1935), Blattidae (Blattodea, BROWN AND NAGAI, 1969), Tipulidae (Diptera, NEUGART et al., 2009). However, no correspondence was found for it on HAO (2021) and therefore the use of this term is suggested to designate it as specified in section 5.3. No variation concerning this muscle was found in this analysis and it seems that this muscle is extremely consistent in insects and variation is likely to be discovered in other taxonomic levels.

7 CONCLUSIONS

The monophyly of the Apidae is highly supported by morphology and DNA data, and here an addition of four new putative synapomorphies for the bees is presented. One additional synapomorphy for the long-tongued bees was also indicated. Among subfamilies, Halictinae has the most peculiar morphology probably related to the remarkable morphological conformation of the labiomaxillary tube of this group when compared to other bees. Apinae, on the other hand, seems to be the subfamily with most variation, probably linked with the age of this clade. As several species of Apinae were selected, a sampling bias should not be discarded. Only one difference allied to sexual dimorphism was found, with Andreninae males with the anterior-cranio mandibular muscle originating in the genal area and in the posterior insertion of the tentorium. Besides variation on the size of mandibular muscles, no differences were found in the musculature of macrocephalic and non-macrocephalic Andreninae.

Some of the features studied here have been demonstrated to have high variation and can further shown to be useful at other taxonomic levels, above and below the family group. Additionally, a standardized terminology for the extrinsic musculature of the group is suggested, aiming to assist future comparative studies for bees and allied groups.

The first comprehensive comparative analysis of the extrinsic musculature of the head of bees using a simple dissection technique is presented. The low-cost technique used here allows it to be replicated in other study groups even with scarce funding or without access to high-tech equipment, something especially relevant in a scenario of modest investments in national science. Investments are important for scientific development and even a simple technique like the one used in this study has behind it a high investment in basic science and free public education. Wipfler et al. (2016) commented that detailed morphological work based on simple dissection was extremely time-consuming and restricted the analysis to one body part and a single species. These criticisms ignore that investigations demand training. The dissections are not time-consuming, instead, they provide time to interpret and understand morphology. After all, every available techniques and resources should be applied for science advance.

REFERENCES

ALAM, S. M. The Skeleto-Muscular Mechanism of *Stenobracon deesae* Cam. (Brae, Hymenoptera)—Ectoparasite of Sugarcane and Jugar Borer of India: I. Head and Thorax. **Aligarh Muslim University Publications**, v. 3, p. 1-74, 1951.

ALEXANDER, B. A.; MICHENER, C.D. Phylogenetic studies of the families of short-tongued bees (Hymenoptera: Apoidea). **University of Kansas Science Bulletin**, v. 55, p. 377-424, 1995.

ASCHER, J. S.; PICKERING, J. Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Available in <http://www.discoverlife.org/mp/20q?guide=Apoidea_species> (accessed on 30 March 2021).

BEUTEL, R. G.; VILHELMSEN, L. Head anatomy of Xyelidae (Hexapoda: Hymenoptera) and phylogenetic implications. **Organisms, Diversity and Evolution**, v. 7, p. 207-230, 2007.

BEUTEL, R. G.; KROGMANN, L.; VILHELMSEN, L. The larval head morphology of *Xyela* sp. (Xyelidae, Hymenoptera) and its phylogenetic implications. **Journal of Zoological Systematics and Evolutionary Research**. v. 46., p. 118-132, 2008.

BEUTEL, R.G. et al. Insect Morphology and Phylogeny: A Textbook for Students of Entomology. **Walter de Gruyter, Berlin**. 2014.

BRANSTETTER, M. G. et al. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. **Curr Biol**, v. 27, p. 1019-25, 2017.

BRAZEAU, M. D. Problematic character coding methods in morphology and their effects. **Biological Journal of the Linnean Society**. v. 104, p. 489-498. 2011.

BROWN, B. E.; NAGAI, T. Insect visceral muscle: Neural relations of the proctodeal muscles of the cockroach. **Journal of Insect Physiology**, 15(10), 1767–1783. 1969.

DAL MOLIN, A. Secagem química de pequenos artrópodes. 2018. Available in <https://dx.doi.org/10.17504/protocols.io.s9aeh2e> (acessed on 30 December 2020).

DANFORTH, B. N. The morphology and behavior of dimorphic males in *Perdita portalis* (Hymenoptera: Andrenidae). **Behav Ecol Sociobiol**. 29, 235–247 1991.

DANFORTH, B. N.; FANG, J.; SIPES, S. Analysis of family-level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear

genes: CAD and RNA polymerase II. **Mol Phylogenet Evol**, v. 39, p. 358-72, 2006.

DANFORTH, B. N. et al. The Impact of Molecular Data on Our Understanding of Bee Phylogeny and Evolution. **Annual Review of Entomology**, v. 58, p. 57-78, 2013.

DE PINNA, M. C. C. Concepts and tests of homology in the cladistic paradigm. **Cladistics.** v. 7, p. 367-394. 1991.

DEBEVEC, A. H.; CARDINAL, S.; DANFORTH, B. N. Identifying the sister group to the bees: a molecular phylogeny of Aculeata with an emphasis on the superfamily Apoidea. **Zoologica Scripta**, v. 41, p. 527–535, 2012.

DUNCAN, C. D. A contribution to the biology of North America vespine wasps. Stanford **Univ. Publ. Biological Sciences**, v. 8, p. 1-272, 1939.

EICKWORT, G. C. A comparative morphological study and generic revision of the augochlorine bees (Hymenoptera: Halictidae). **Univ. Kansas Sci. Bull**, v. 48, p. 325-524, 1969.

GRAF, V. Contribuição ao estudo da anatomia da cabeça dos Apoidea. - A musculatura do complexo lábio-maxilar de Xylocopa Latreille, 1802 (Hymenoptera-Apoidea). **Boletim da Universidade Federal do Paraná, Zoologia**. v. 2(7): p. 93-100. 1965.

GRAF, V. Contribuição ao estudo da anatomia da cabeça dos Apoidea II - A musculatura do complexo lábio - maxilar. **Boletim da Universidade Federal do Paraná, Zoologia**. v. 5: p. 139-173. 1972.

HADLEY, A. Combine ZM software, new version. 2010.

HAMILTON, W. D. Wingless and fighting males in fig wasps and other insects. In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition in insects. **Academic Press, New York**, pp 167–220. 1979.

HEDTKE, S. M.; PATINY, S.; DANFORTH, B. N. The bee tree of life: a supermatrix approach to apoid phylogeny and biogeography. **BMC Evol. Biol**., v.13, 2013.

HERATY, J. M.; WOOLLEY, J. B.; DARLING, D. C. Phylogenetic implications of the mesofurca and mesopostnotum in Hymenoptera. **Journal of Hymenoptera** Research, v. 3, p. 241-277, 1994.

HERATY, J. M. et al. Evolution of the hymenopteran megaradiation. **Molecular Phylogenetics and Evolution**, v. 60, p. 73-88. 2011.

HOUSTON, T. F. Discovery of an apparent male soldier caste in a nest of a halictine bee (Hymenoptera: Halictidae) with notes on the nest. **Aust J Zool**. 18:345-351. 1970.

HUSEMANN, M. et al. Mitogenomic phylogeny of bee families confirms the basal position and monophyly of Melittidae. **Zool Scr**, v. 00, p. 1- 6. 2021.

JOSEPHSON, R. Muscle System. **Encyclopedia of Insects**. 675 - 680. 2009.

KLOPFSTEIN, S. et al. The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data. **PLoS One**, v. 8, p. 1-23, 2013.

KUKUK, P. F.; SCHWARZ, M. Intranest behavior of the communal sweat bee *Lasioglossum* (*Chilalictus*) *erythrurum* (Hymenoptera: Halictidae). **J Kans Entomol Soc.** 60:58–64. 1987.

KUKUK, P. F.; M. P. SCHWARZ. Macrocephalic male bees as functional reproductives and probable guards. **Pan-Pacific Entomologist** 64:131–137. 1988.

MADDISON, W. P.; MADDISON, D. R. Mesquite: a modular system for evolutionary analysis. Version 3.61. http://www.mesquiteproject.org. 2019.

MATSUDA, R. Morphology of the Head of a Sawfly, *Macrophya pluricincta* Norton (**Hymenoptera**, Tenthredinidae). **Journal of the Kansas Entomological Society**, v. 30, p. 99-107, 1957.

MATSUDA, R. Morphology and Evolution of the Insect Head. **The American Entomological Institute, Ann Arbor**, p. 1-344, 1965.

MEIRA, O. M.; GONÇALVES, R. B. The relevance of the mesosomal internal structures to the phylogeny of Augochlorini bees (Hymenoptera: Halictinae). **Zoologica Scripta**, v. 47, p. 197–205, 2018.

MELO, G. A. R. Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps. **Scientific Papers, Natural History Museum, the University of Kansas**, v. 14, p. 1–55, 1999.

MELO, G. A. R.; GONÇALVES, R. B. Higher-level bee classifications (Hymenoptera, Apoidea, Apidae *sensu lato*). **Revista Brasileira de Zoologia**, v. 22, p. 153-159, 2005.

MICHENER, C. D. Comparative external morphology, phylogeny, and a classification of the bees. **Am. Museum Nat. Hist. Bult**, v. 82, p. 151-326, 1944.

MICHENER, C. D. Comparative morphology of the middle coxae of Apoidea. **Journal of the Kansas Entomological Society**, v. 54, p. 319-326, 1981.

MICHENER, C. D. The bees of the world. **Baltimore, MD: John Hopkins University Press**. 2007.

MIKÓ, I. et al. Skeletomusculature of Scelionidae (Hymenoptera: Platygastroidea): head and mesosoma. **Zootaxa**, v. 1571, p. 1-78, 2007.

MORISON, G. D. The Musculature of the Adult Honey Bee (*Apis mellifera*): Parts I and II. **Quarterly Journal of Microscopical Science**, v. 71, p. 395-463, p. 563-631, 1927.

NEUGART, C.; SCHNEEBERG, K.; BEUTEL, R. G. The morphology of the larval head of Tipulidae (Diptera, Insecta) – the dipteran groundplan and evolutionary trends. **Zool. Anz**, v. 248, p. 215-235, 2009.

NIXON, K. WinClada, Version 1.00.08. Published by the Author, Ithaca, NY. 2002.

OHL, M.; BLEIDORN, C. The phylogenetic position of the enigmatic wasp family Heterogynaidae based on molecular data, with description of new nocturnal species. **Systematic Entomology**, 2006.

OHL, M.; SPAHN, P. A cladistic analysis of the cockroach wasps based on morphological data (Hymenoptera: Ampulicidae). **Cladistics**, v. 26, p. 49-61, 2010.

PATTERSON, C. Morphological characters and homology. In: K. A. Joysey & A. E. Friday (eds.). Problems in Phylogenetic Reconstruction. **Academic Press, London**, p. 21-74. 1982.

PETERS, R. S. et al. Evolutionary history of the Hymenoptera. **Curr Biol**, v. 27, p. 1013-8, 2017.

PLANT, J.; PAULUS, H. F. Evolution and Phylogeny of Bees - Review and Cladistic Analysis in Light of Morphological Evidence (Hymenoptera, Apoidea) **Zoologica**. v. 161: p. 232. 2016.

PORTO, D. S.; ALMEIDA, E. A. B; VILHELMSEN, L. Comparative morphology of internal structures of the mesosoma of bees with an emphasis on the corbiculate clade (Apidae: Apini). **Zoological Journal of the Linnean Society**, v. 41, p. 339-368, 2016a.

PORTO, D. S.; VILHELMSEN, L.; ALMEIDA, E. A. B. Comparative morphology of the mandibles and head structures of corbiculate bees (Hymenoptera: Apidae: Apini). **Systematic Entomology**, v. 41, p. 339-368, 2016b.

PORTO, D. S.; ALMEIDA, E. A. B. A comparative study of the pharyngeal plate of Apoidea (Hymenoptera: Aculeata), with implications for the understanding of

phylogenetic relationships of bees. – **Arthropod Structure & Development**, v. 50, p. 64-77, 2019.

PRENTICE, M. A. The Comparative Morphology and Phylogeny of Apoid Wasps (Hymenoptera: Apoidea). **Dissertation Thesis. University of California, Berkeley**, 1998.

RICHTER, A. et al. Comparative analysis of worker head anatomy of *Formica* and *Brachyponera* (Formicidae, Hymenoptera, Insecta). **Arthropod Structure & Development**, v. 78, p. 133-170, 2019.

ROIG-ALSINA, A.; MICHENER, C. D. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). **University of Kansas Science Bulletin**, v. 55, p. 123-160, 1993.

SANN, M. et al. Phylogenomic analysis of Apoidea sheds new light on the sister group of bees. **BMC Evolutionary Biology**, v. 18, p. 71, 2018.

SERENO, P. C. Logical basis for morphological characters in phylogenetics. **Cladistics**, v. 23, p. 565-587. 2007.

SNODGRASS, R. E. Morphology and evolution of the insect head and its appendages. **Smithson. Misc. Coll**, v. 81, p. 1-157, 1928.

SNODGRASS, R. E. Principles of insect morphology. **McGraw-Hill, New York, USA**. 1935.

SNODGRASS, R. E. The skeletomuscular mechanism of the honey bee. **Smithson. misc. Collns**, v. 103, p. 1-120, 1942.

URBAN, D. Estudo comparativo da origem de alguns músculos cefálicos das abelhas (Hymenoptera-Apoidea). **Boletim da Universidade Federal do Paraná, Zoologia**. v. 2(7): p. 21-33. 1963.

VILHELMSEN, L. The preoral cavity of lower Hymenoptera (Insecta): comparative morphology and phylogenetic significance. **Zoologica Scripta**, v. 25, p. 143-170, 1996.

VILHELMSEN L.; MIKÓ I.; KROGMANN L. Beyond the wasp-waist: structural diversity and phylogenetic significance of the mesosoma in apocritan wasps (Insecta: Hymenoptera). **Zool. J. Linn. Soc**., v. 159, p. 22-194, 2010.

ZIMMERMANN, D.; VILHELMSEN, L. The sister group of Aculeata (Hymenoptera) – evidence from internal head anatomy, with emphasis on the tentorium. **Arthropod Systematics and Phylogeny**. 74. 195-218. 2016.

WIPFLER, B. et al. On the head morphology of Grylloblattodea (Insecta) and the systematic position of the order, with a new nomenclature for the head muscles of Dicondylia. **Systematic Entomology**, 36: 241-266. 2011.

WIPFLER, B. et al. A review of methods for analyzing insect structures - the role of morphology in the age of phylogenomics. **Curr Opin** Insect Sci. 18:60-68. 2016.

WOLFF, O. J. B. Das Riechorgan der Biene. **Nova Acta Leopoldina. Deutsche Akademie der Naturforscher**, v. 38, p.1-251. 1875.

YODER, M. J. et al. A Gross Anatomy Ontology for Hymenoptera. **PloS One**, v. 5, p. 1-8, 2010.

YOUSSEF, N. N. Topography of the cephalic musculature and nervous system of the honey bee *Apis mellifera* Linnaeus. **Smithson. Contrib. Zool**., 99, p. 1-54, 1971.

APPENDIX A - DETAILED ILLUSTRATIONS OF THE STUDY GROUP.

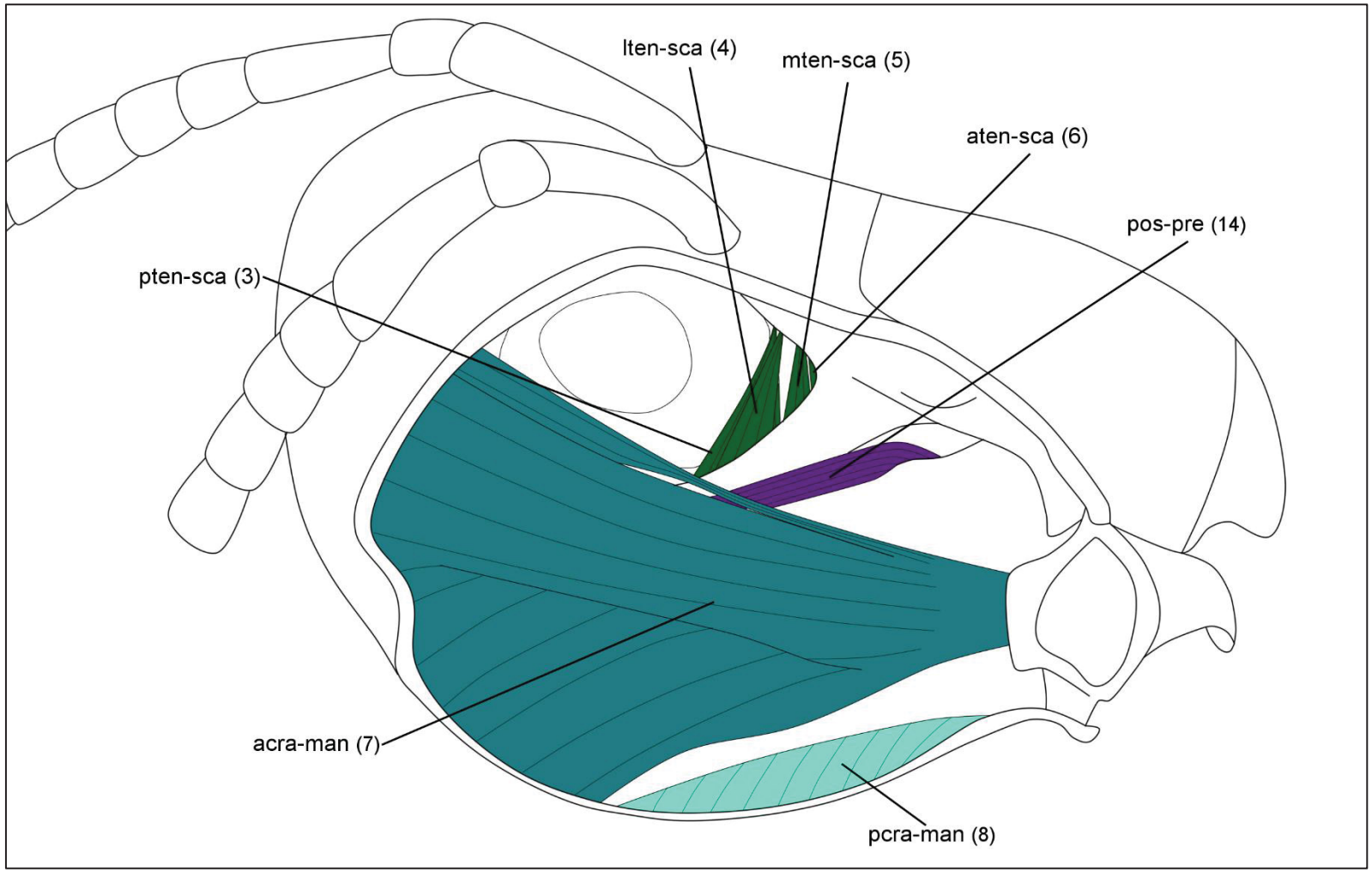

FIGURE 9. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ANDRENA* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

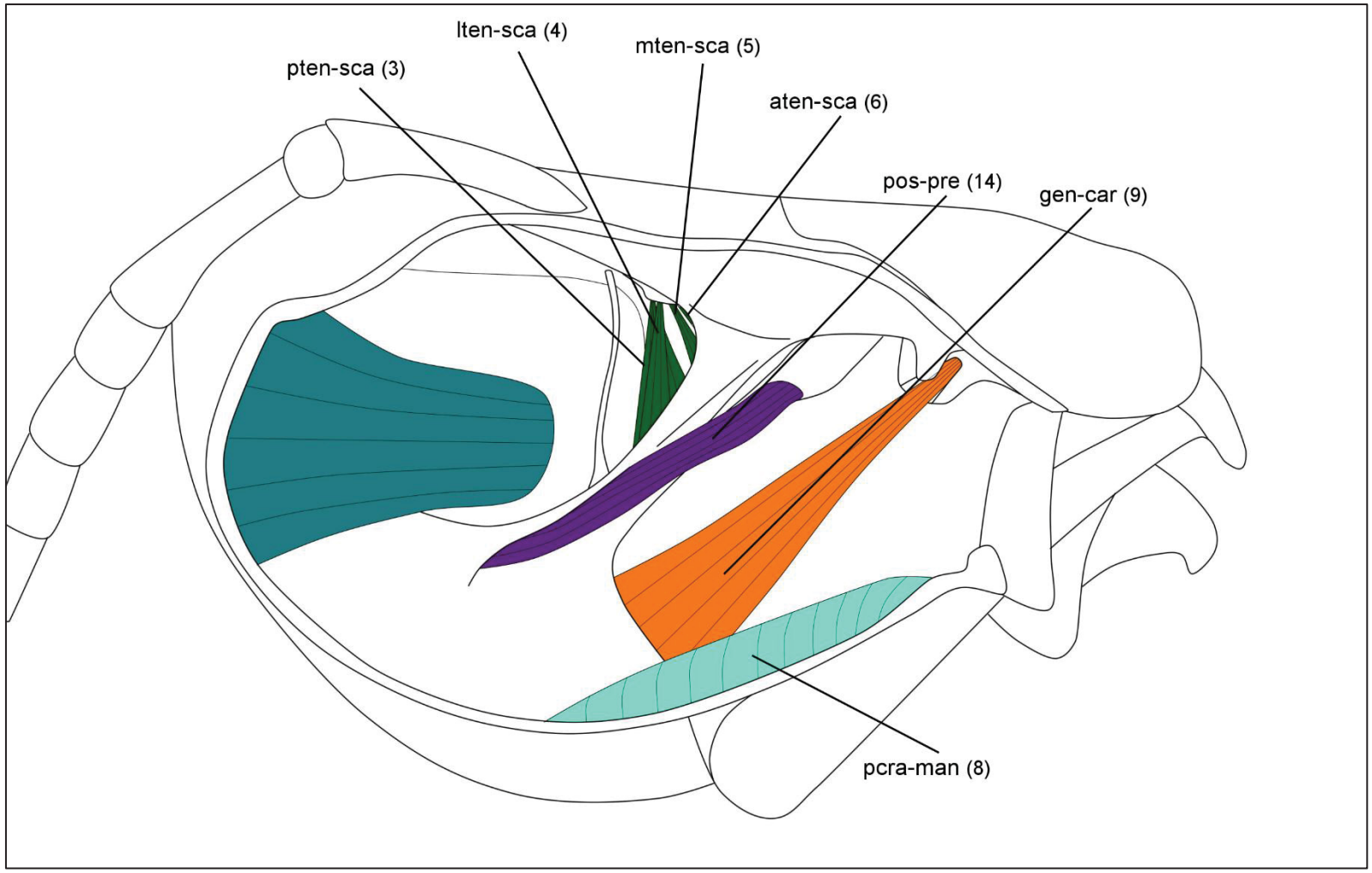

FIGURE 10. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ANDRENA* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

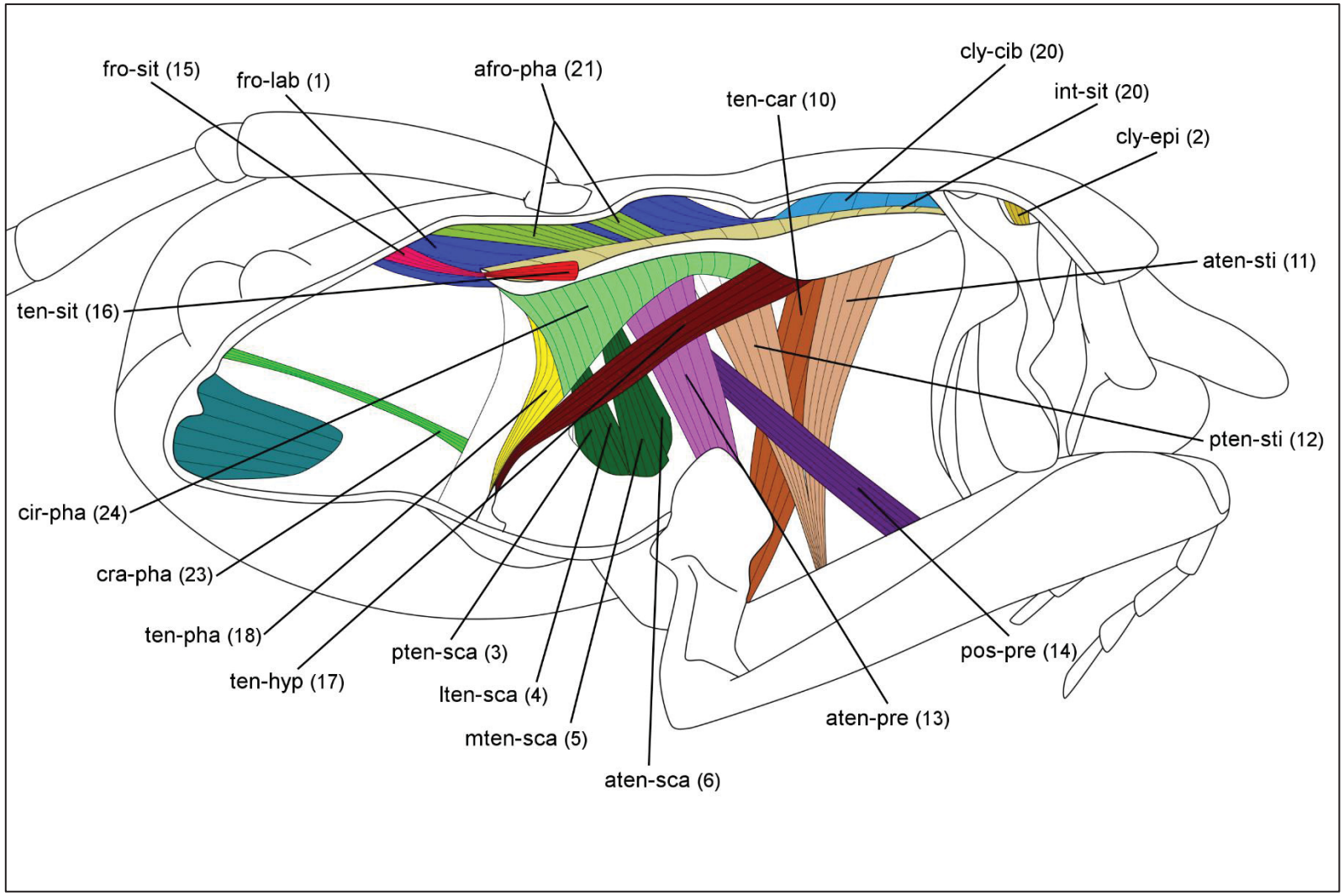

FIGURE 11. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ANDRENA* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

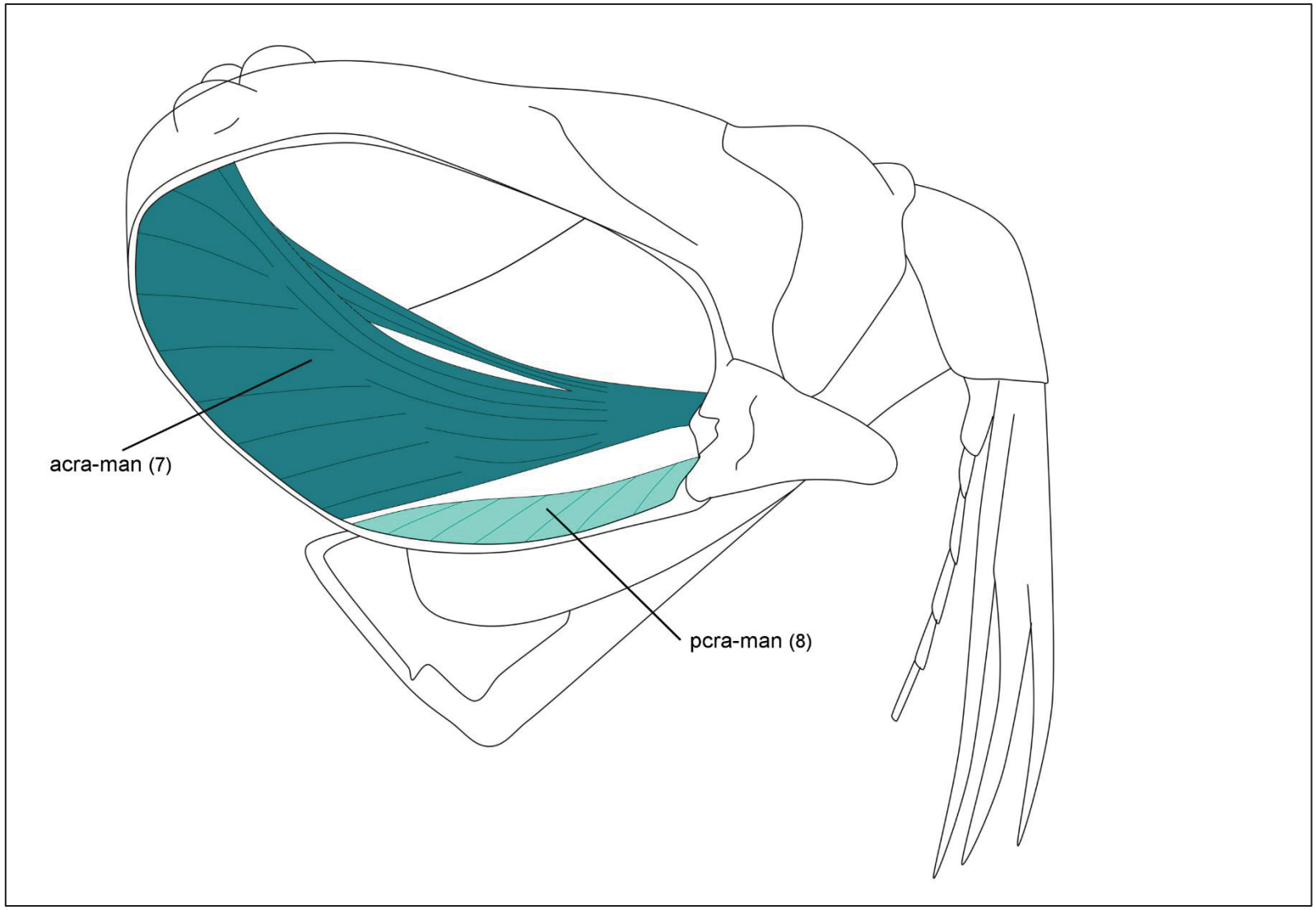

FIGURE 12. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

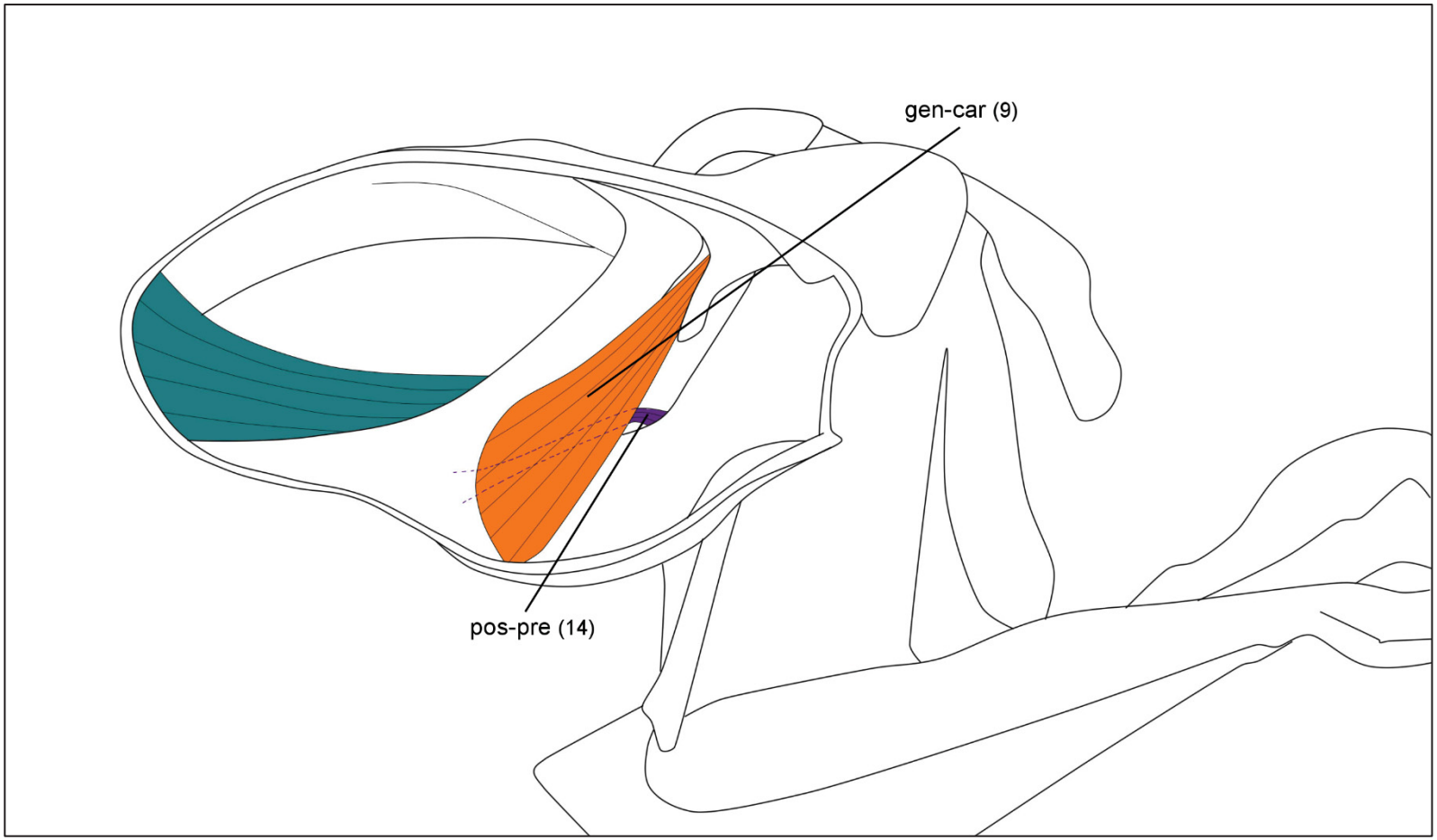

FIGURE 13. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

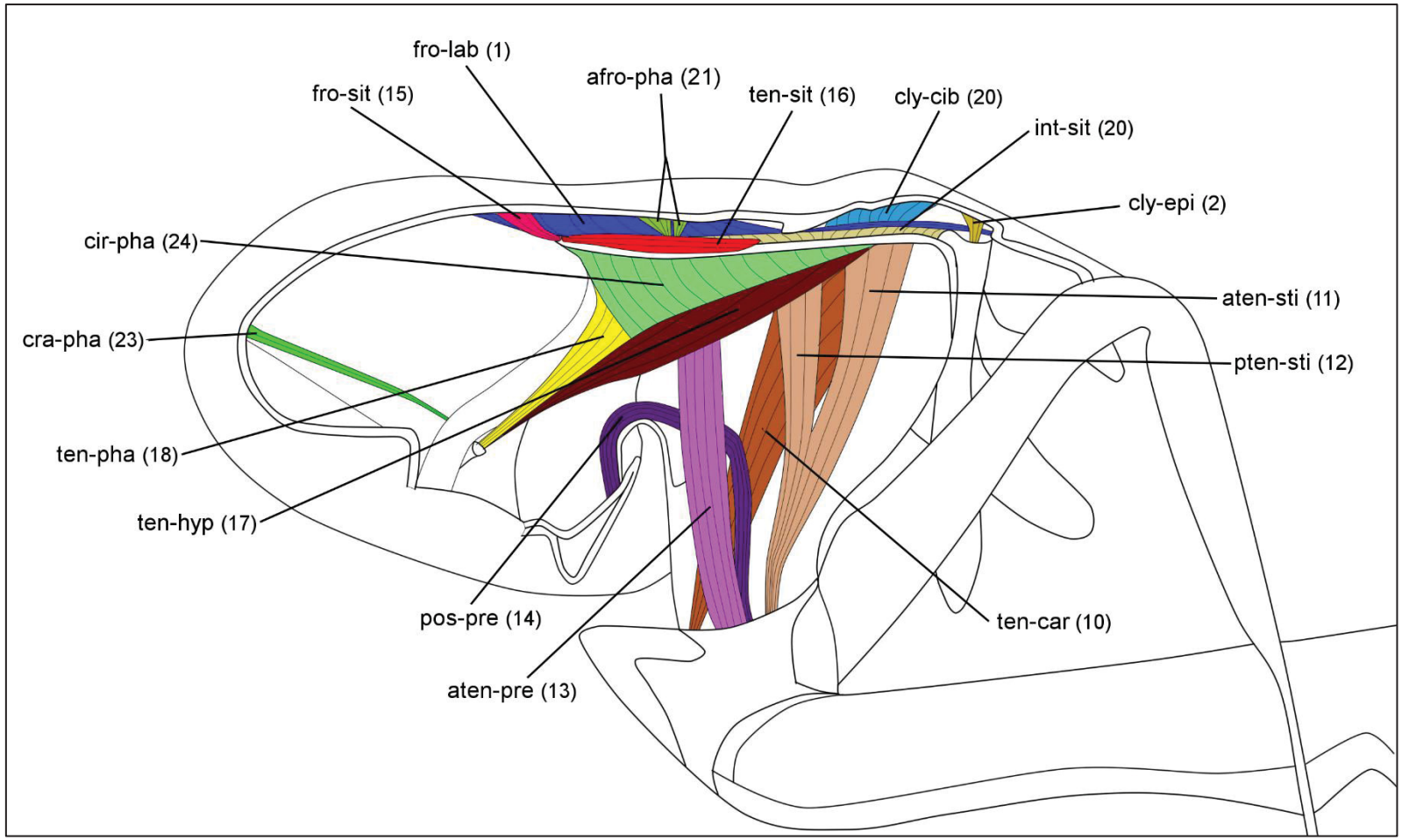

FIGURE 14. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

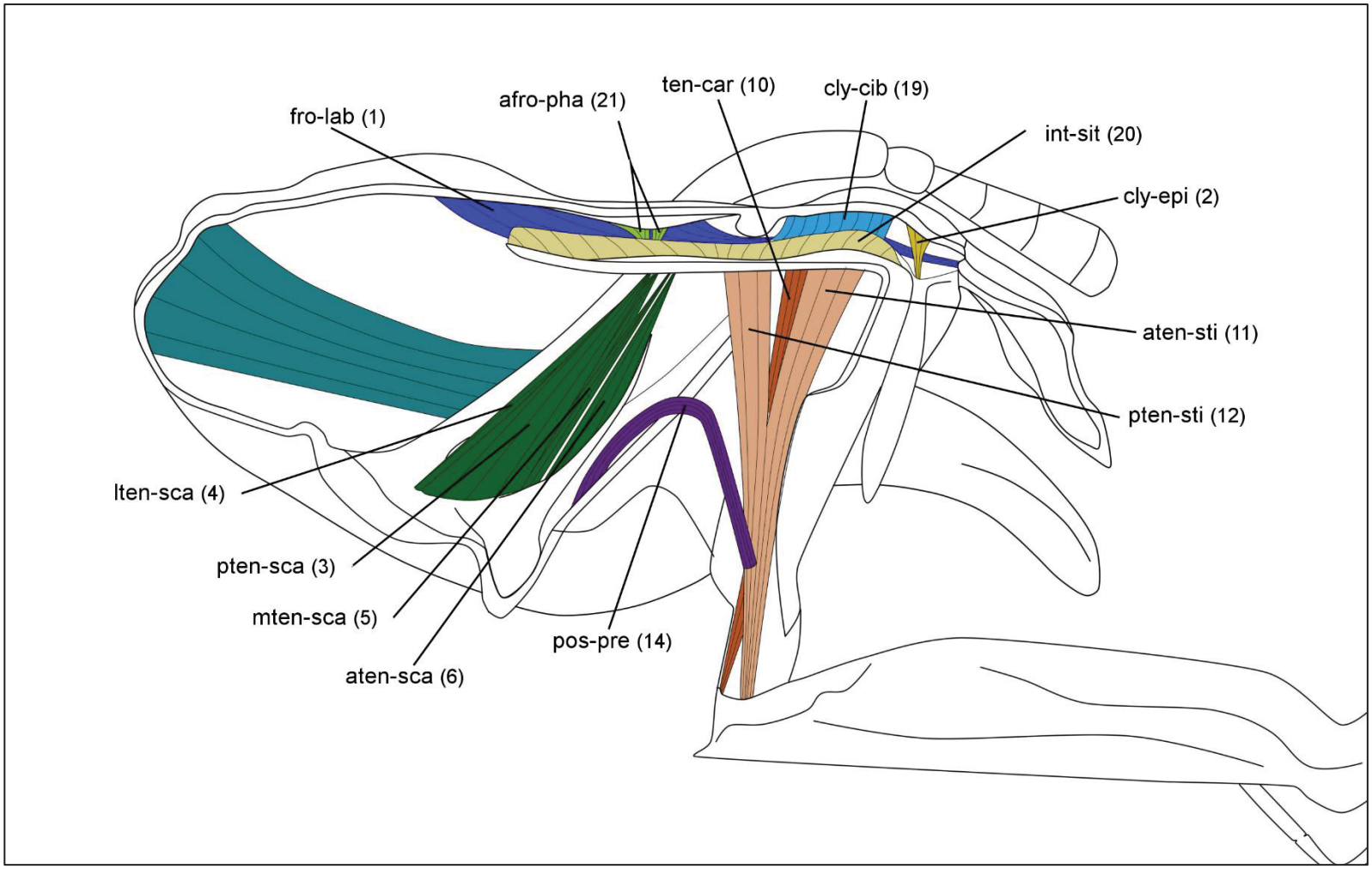

FIGURE 15. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

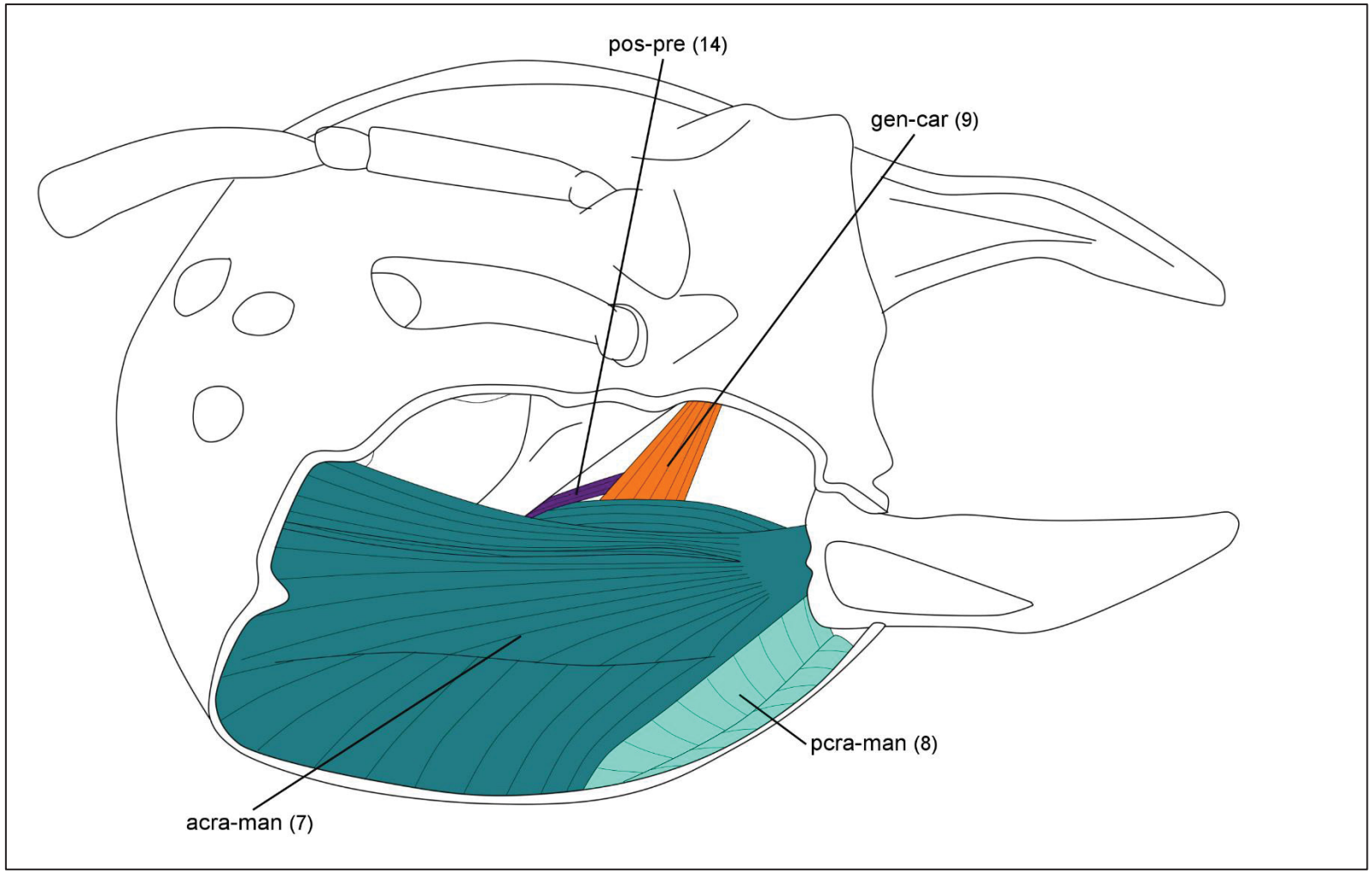

FIGURE 16. EXTRINSIC MUSCLES OF THE HEAD OF MALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

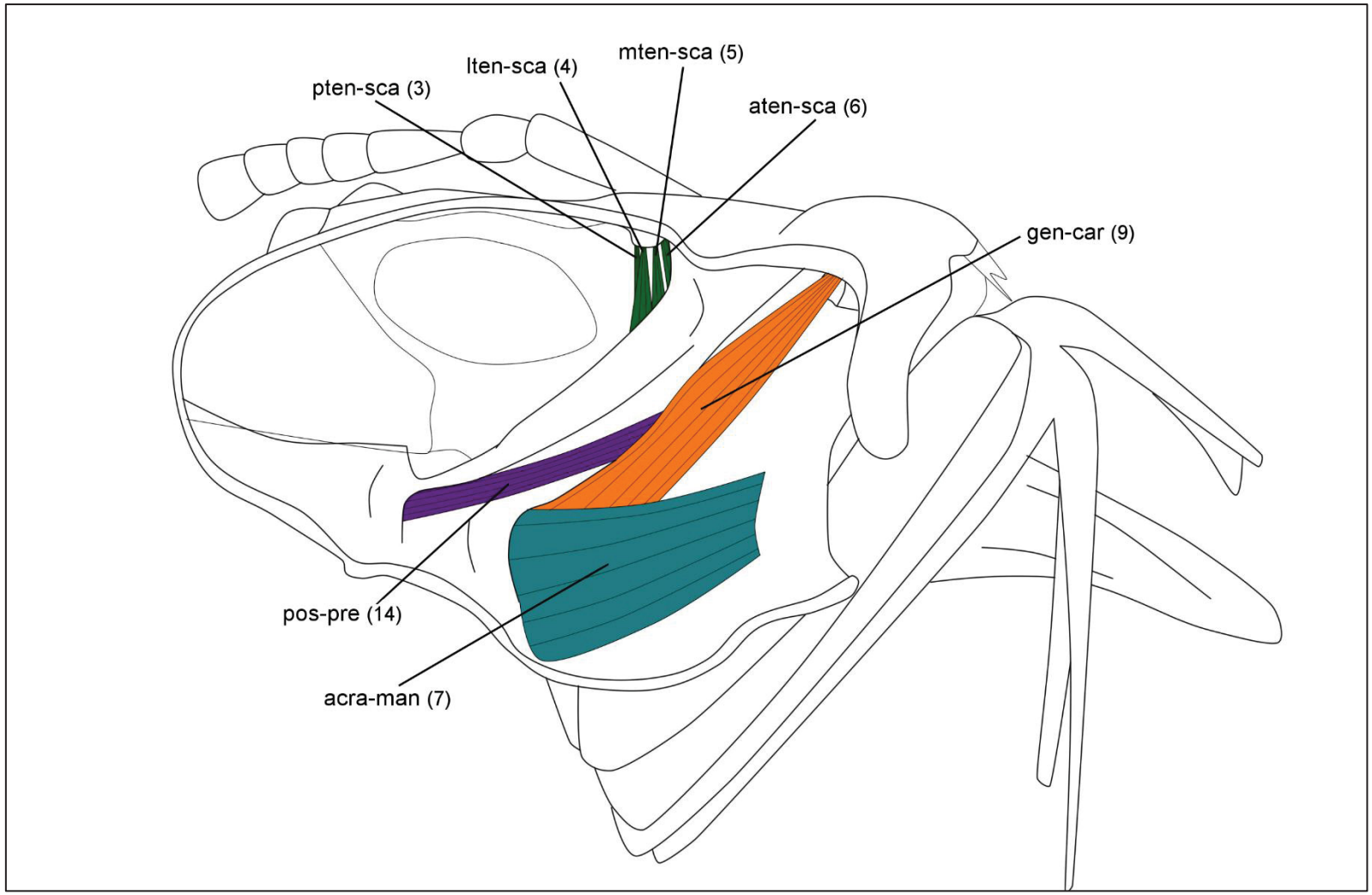

FIGURE 17. EXTRINSIC MUSCLES OF THE HEAD OF MALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

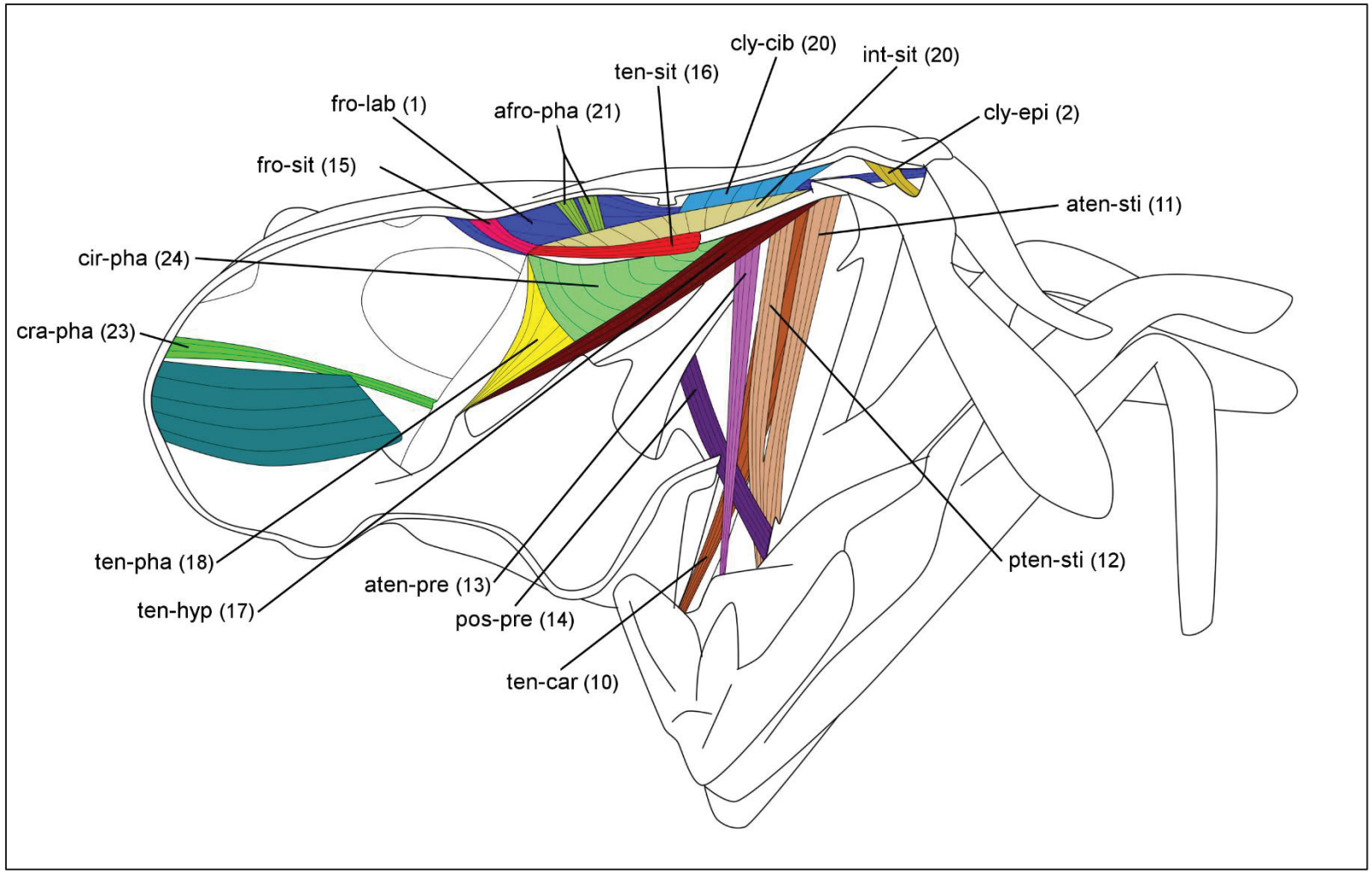

FIGURE 18. EXTRINSIC MUSCLES OF THE HEAD OF MALE *CALLONYCHIUM (CALLONYCHIUM) PETUNIAE* CURE & WITTMANN, 1990 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

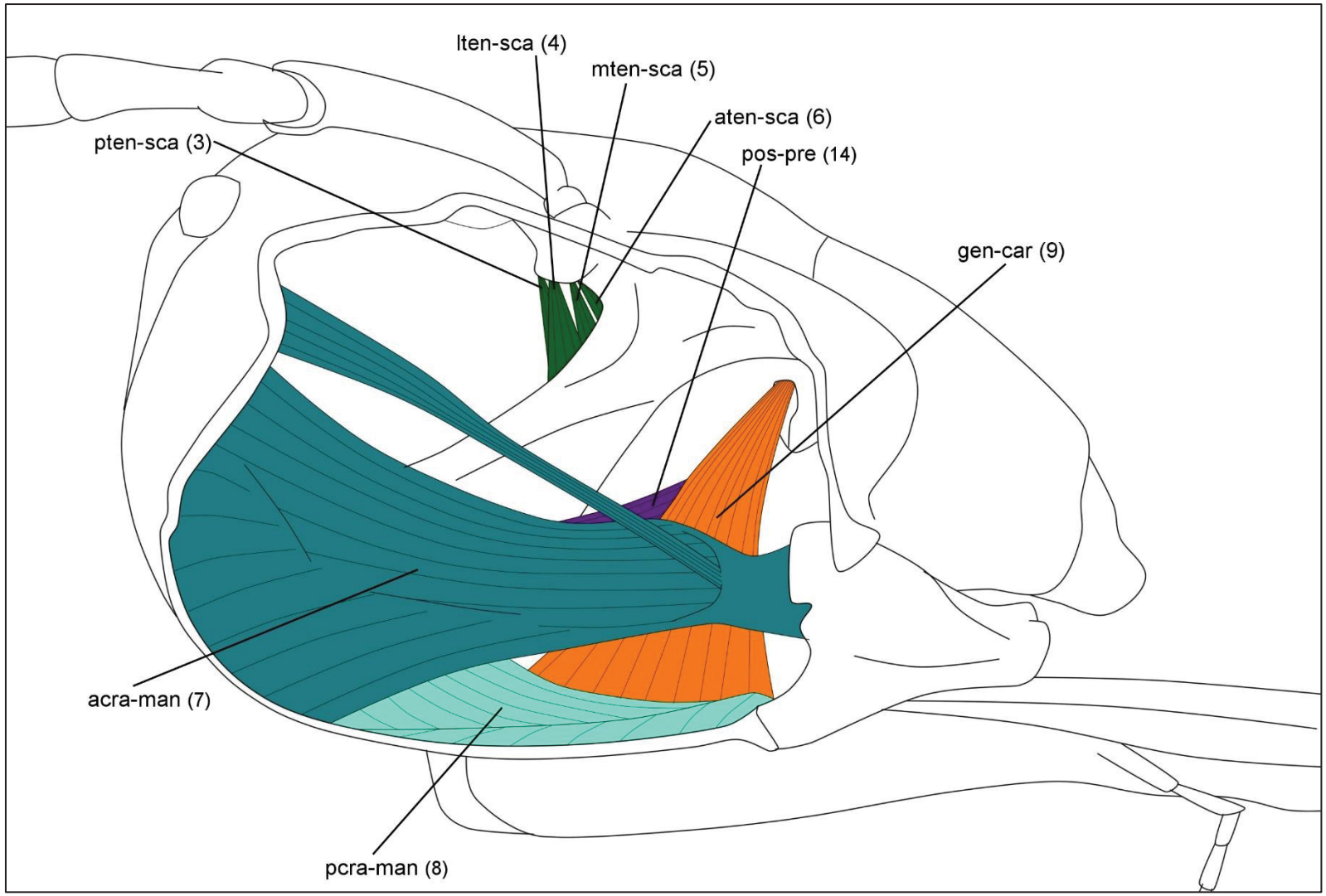

FIGURE 19. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

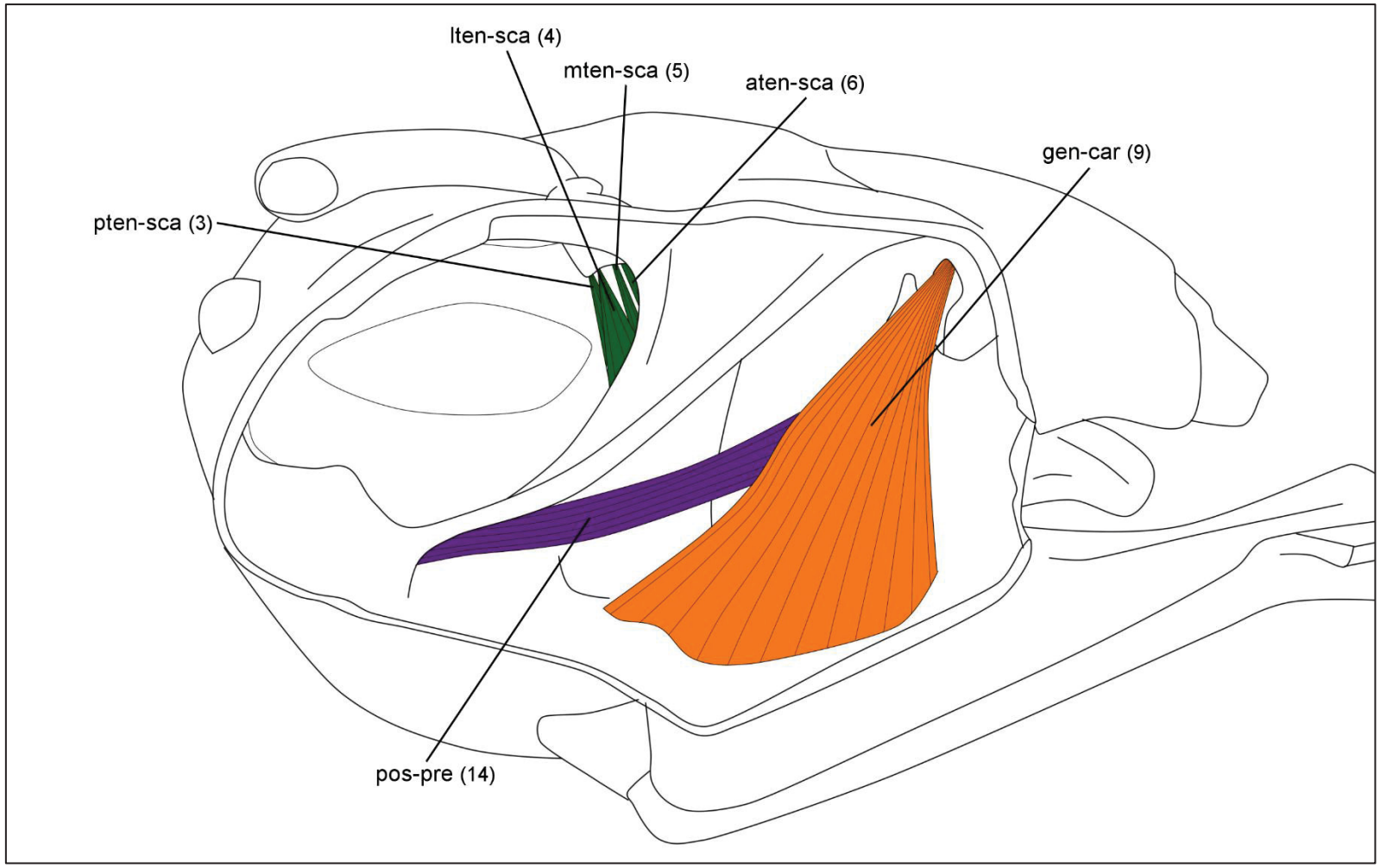

FIGURE 20. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

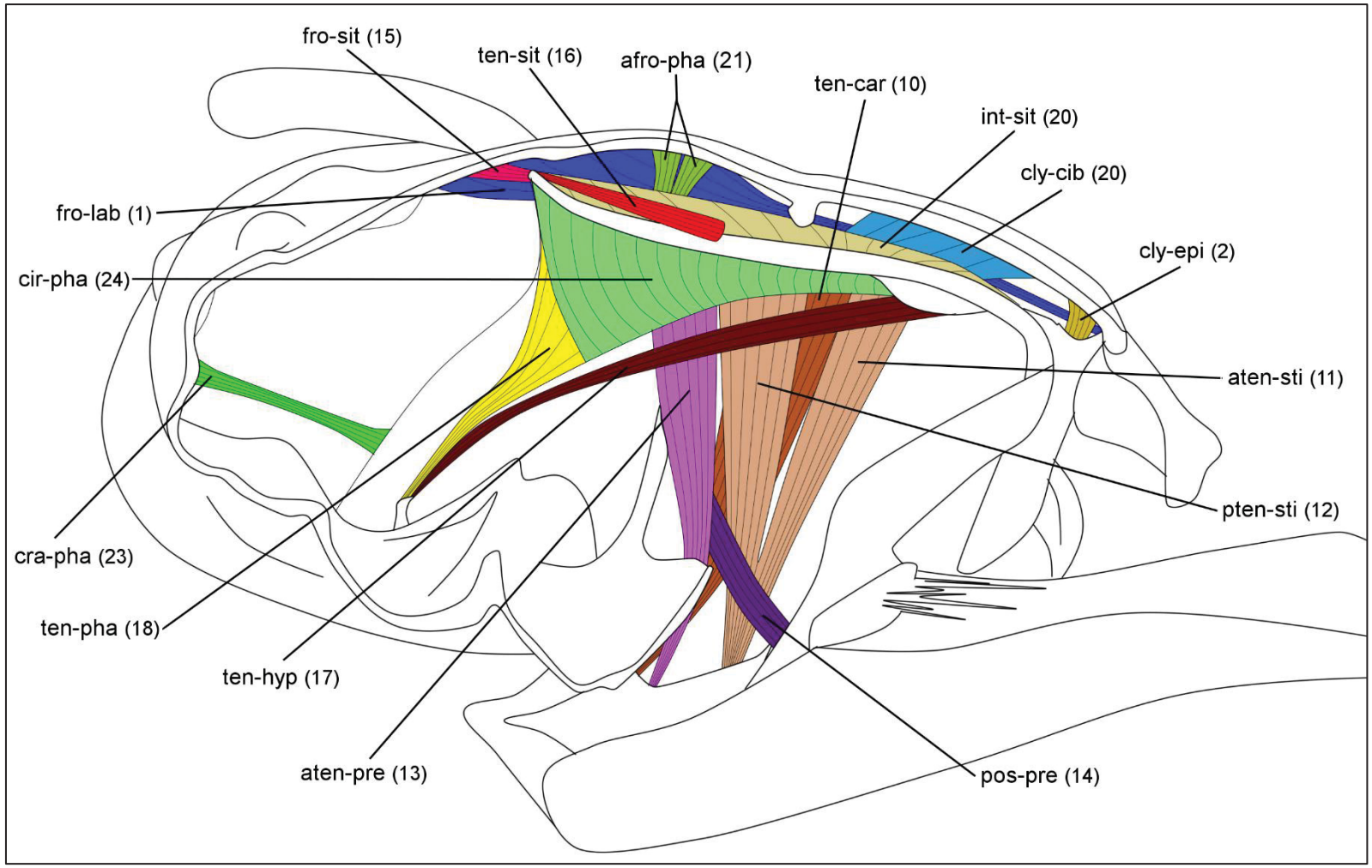

FIGURE 21. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

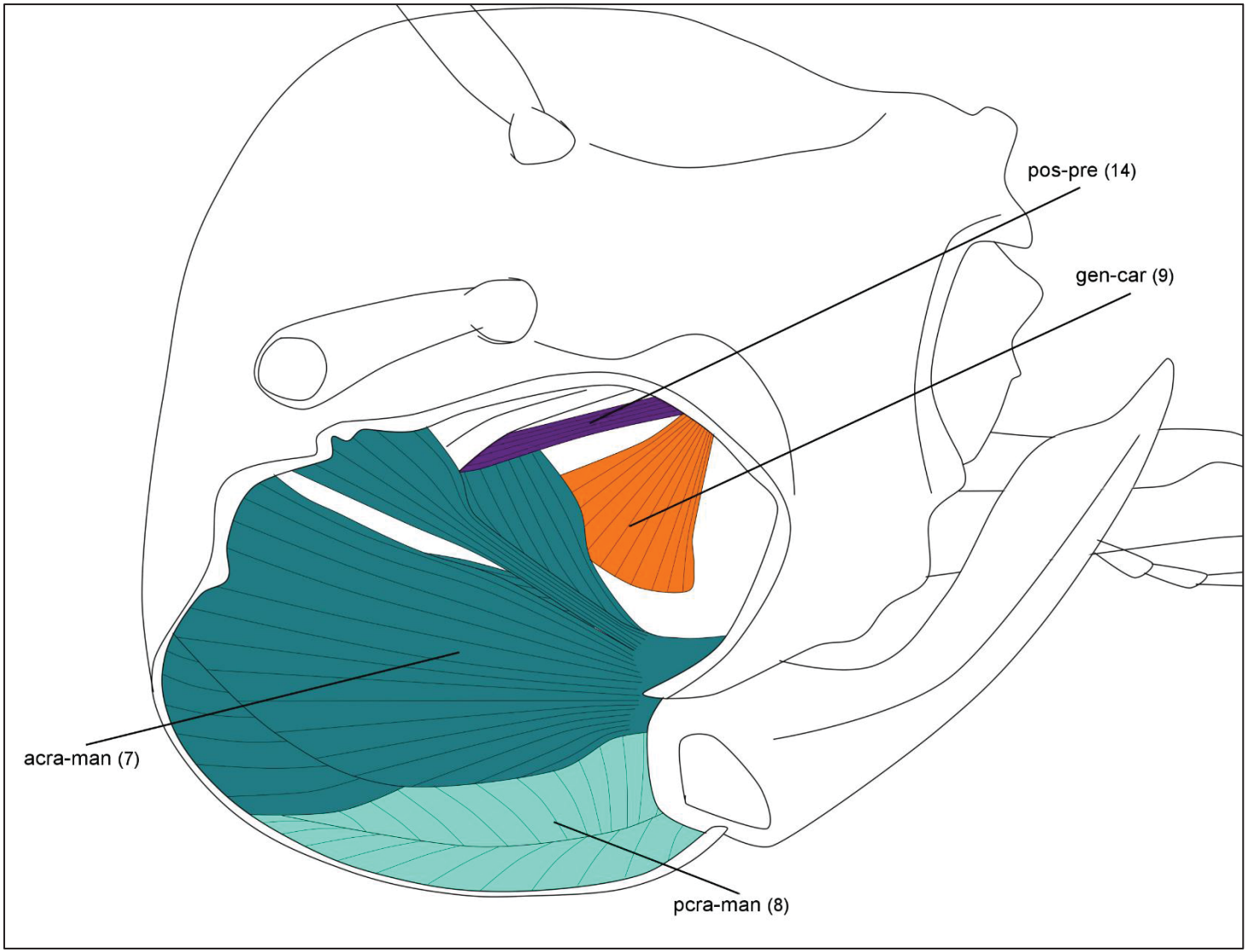

FIGURE 22. EXTRINSIC MUSCLES OF THE HEAD OF MACROCEFALIC MALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

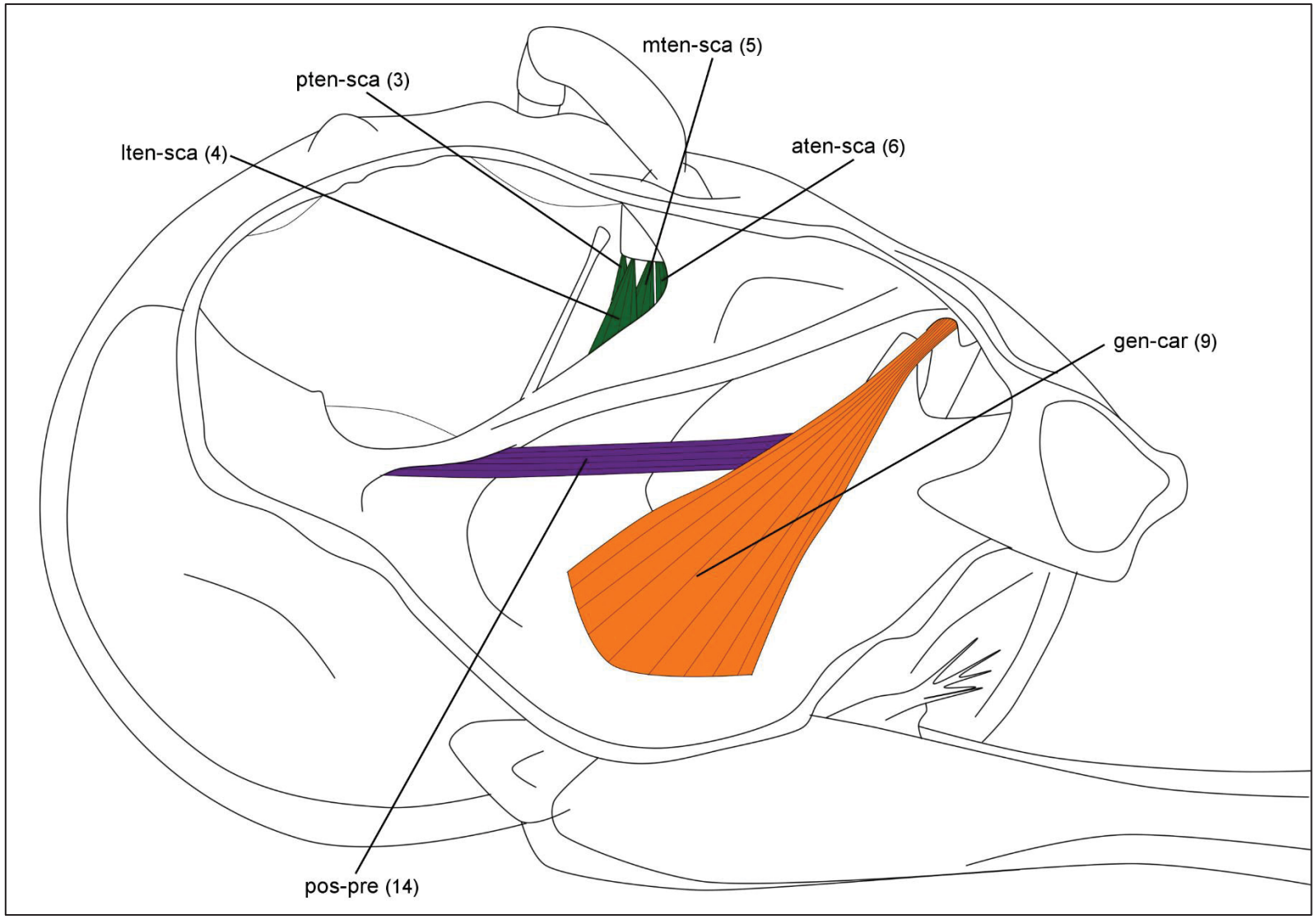

FIGURE 23. EXTRINSIC MUSCLES OF THE HEAD OF MACROCEFALIC MALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

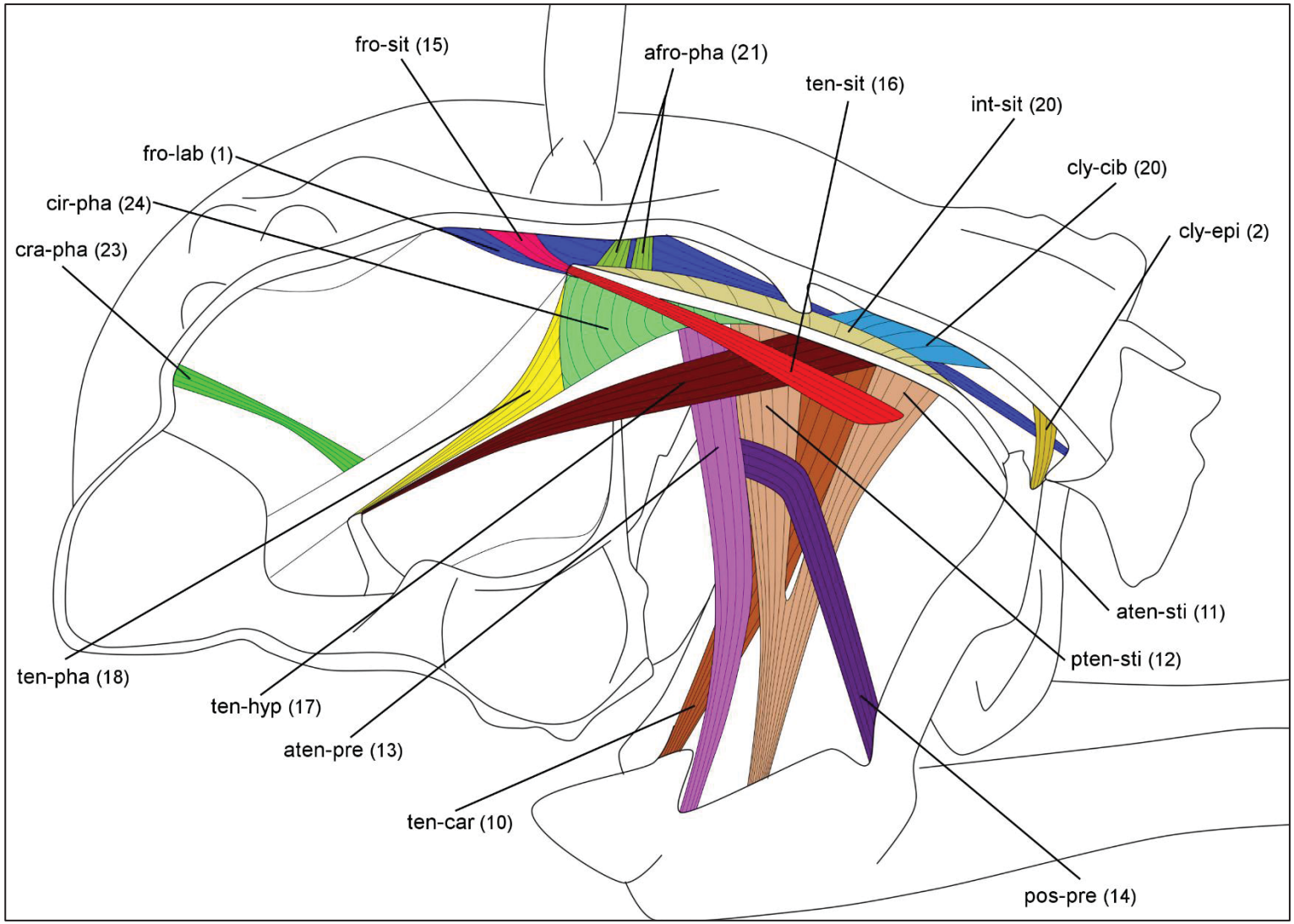

FIGURE 24. EXTRINSIC MUSCLES OF THE HEAD OF MACROCEFALIC MALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

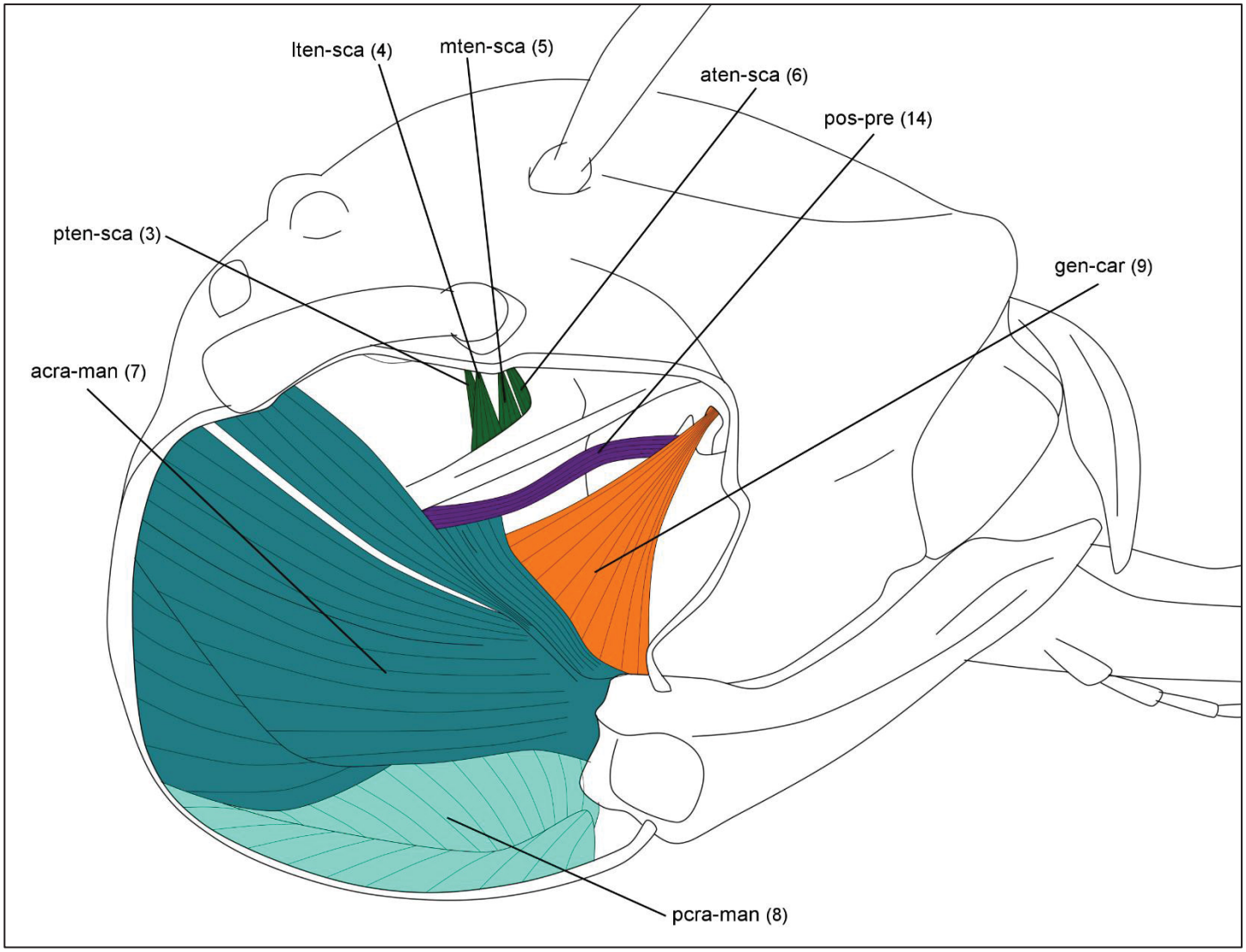

FIGURE 25. EXTRINSIC MUSCLES OF THE HEAD OF NONMACROCEFALIC MALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

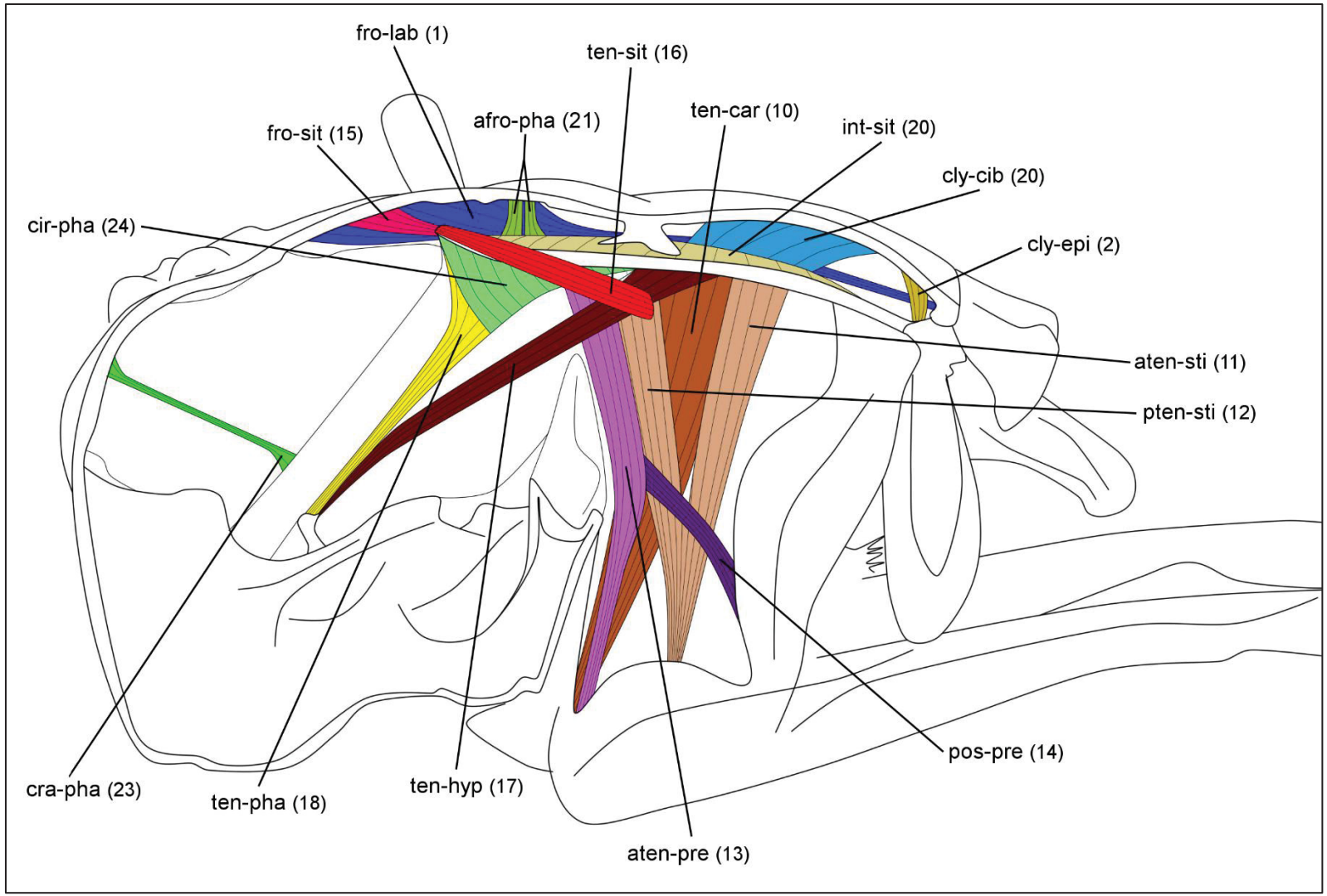

FIGURE 26. EXTRINSIC MUSCLES OF THE HEAD OF NONMACROCEFALIC MALE *PSAENYTHIA ANNULATA* GERSTAECKER, 1868 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

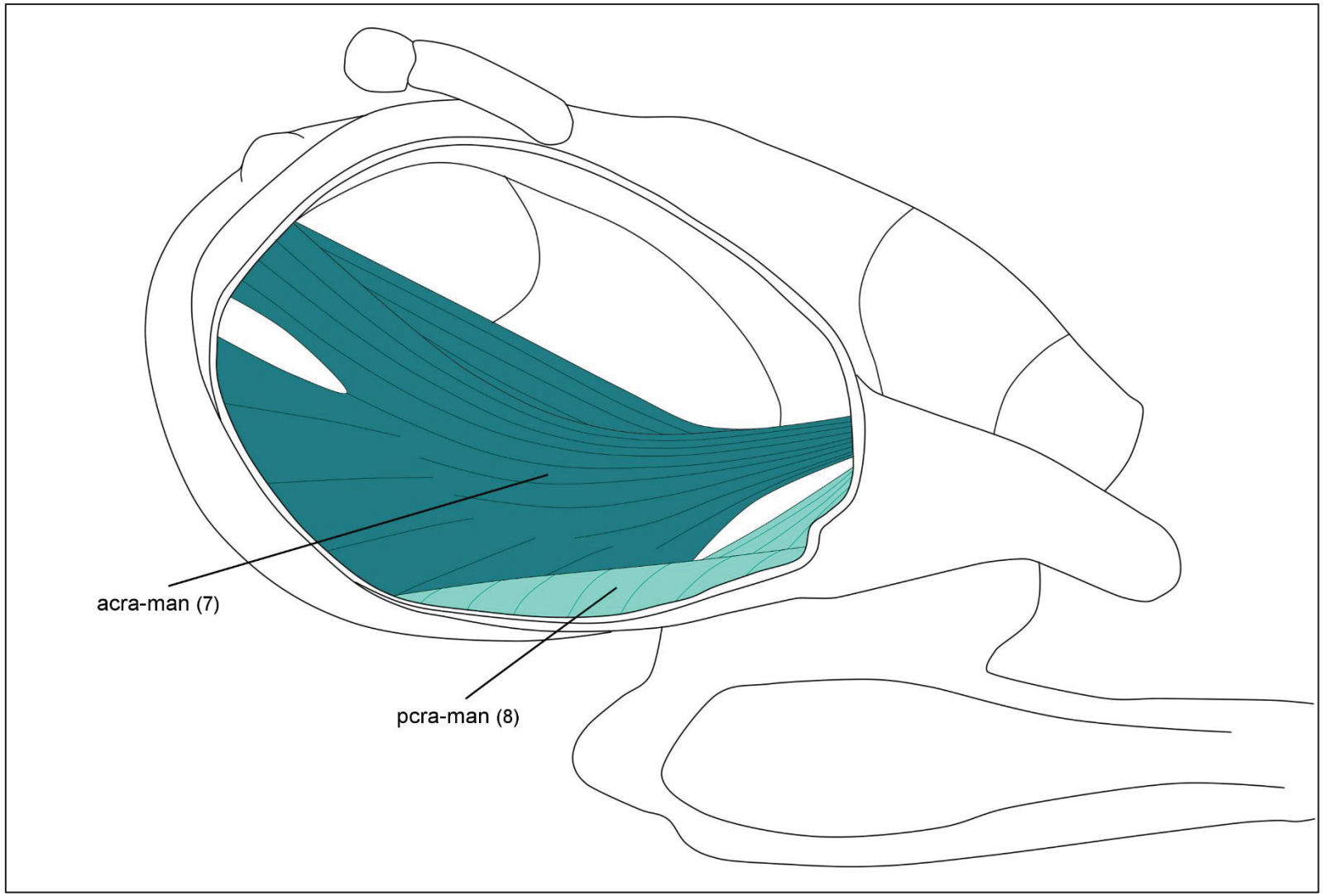

FIGURE 27. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

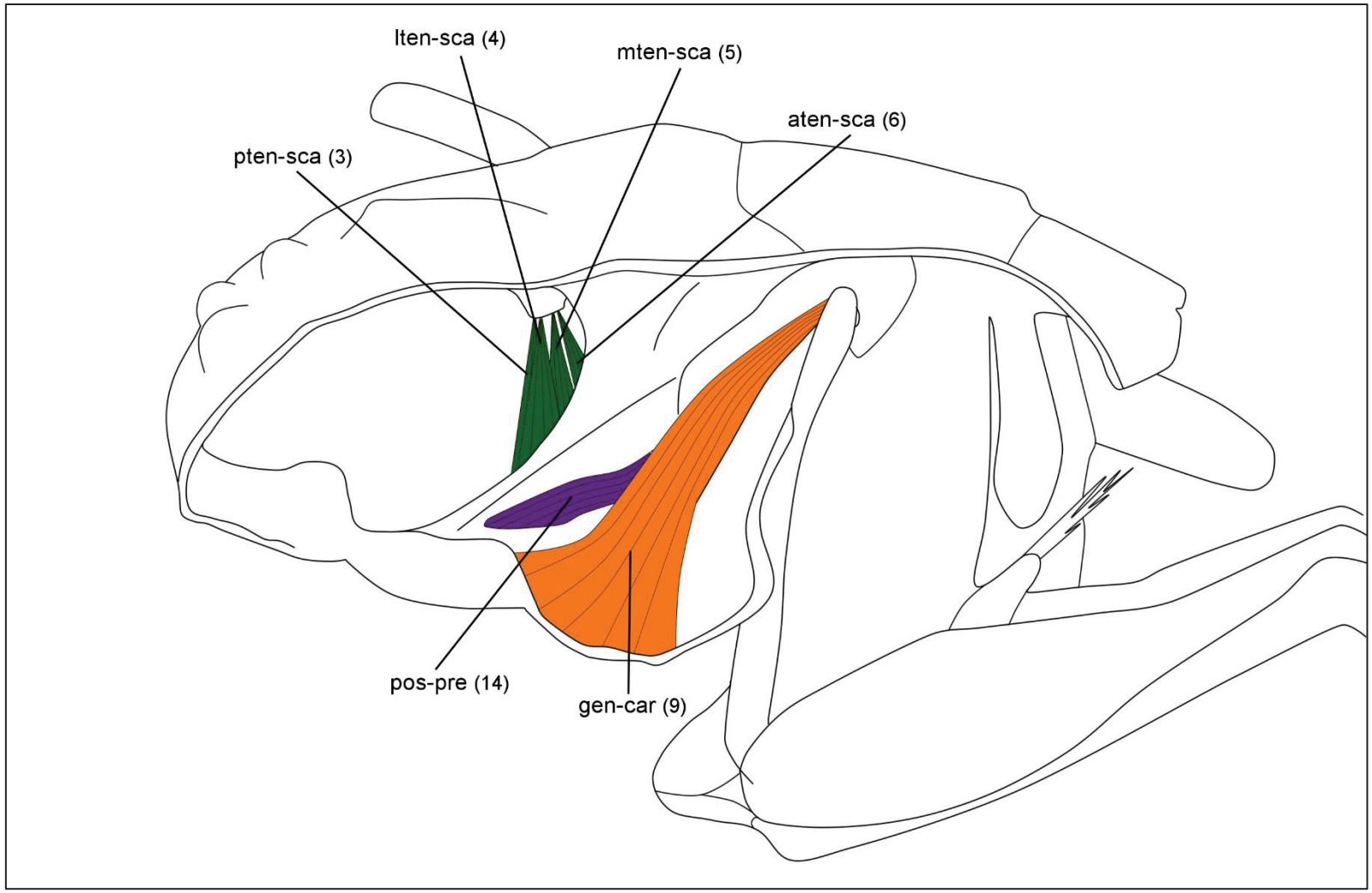

FIGURE 28. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

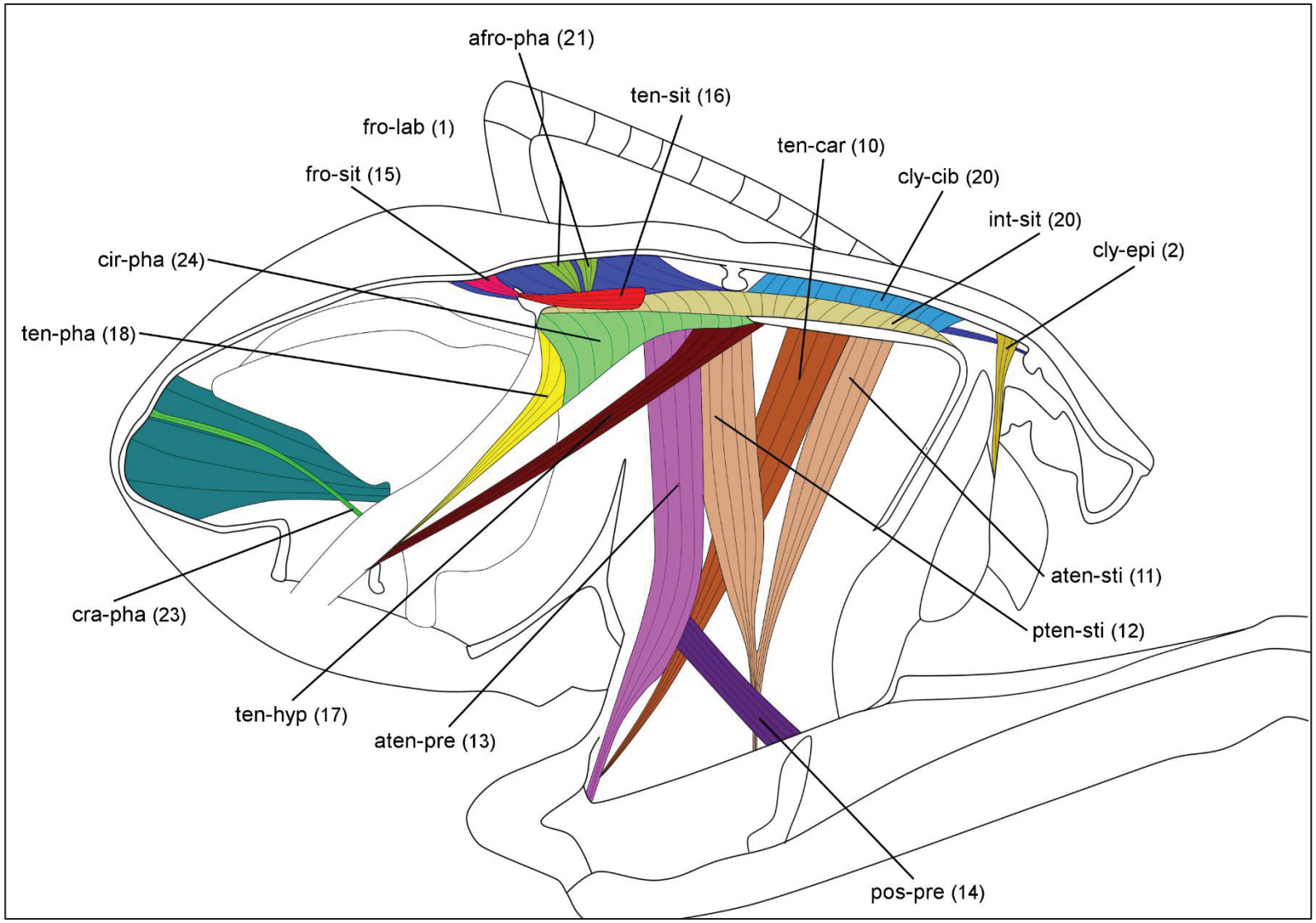

FIGURE 29. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

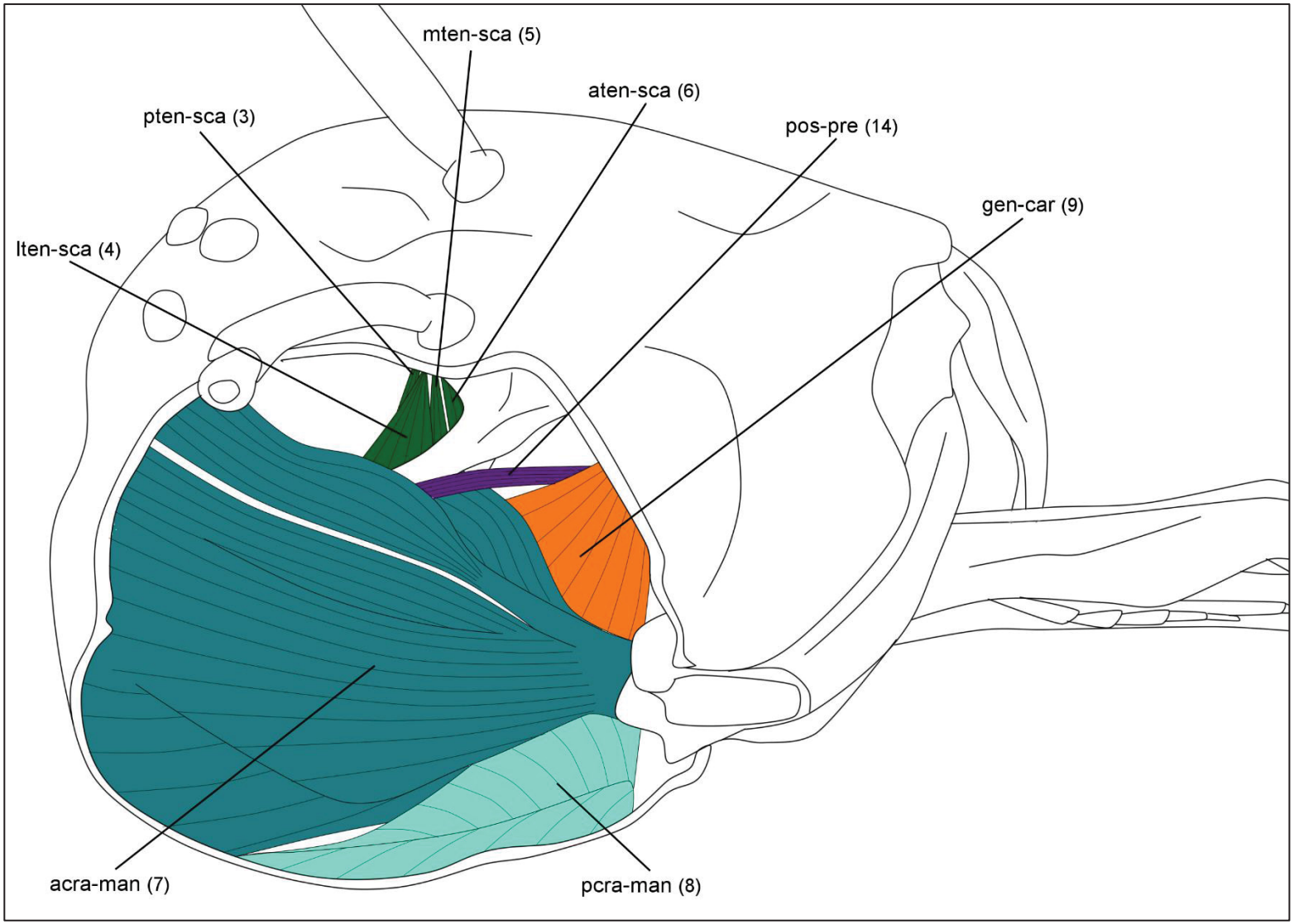

FIGURE 30. EXTRINSIC MUSCLES OF THE HEAD OF MALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

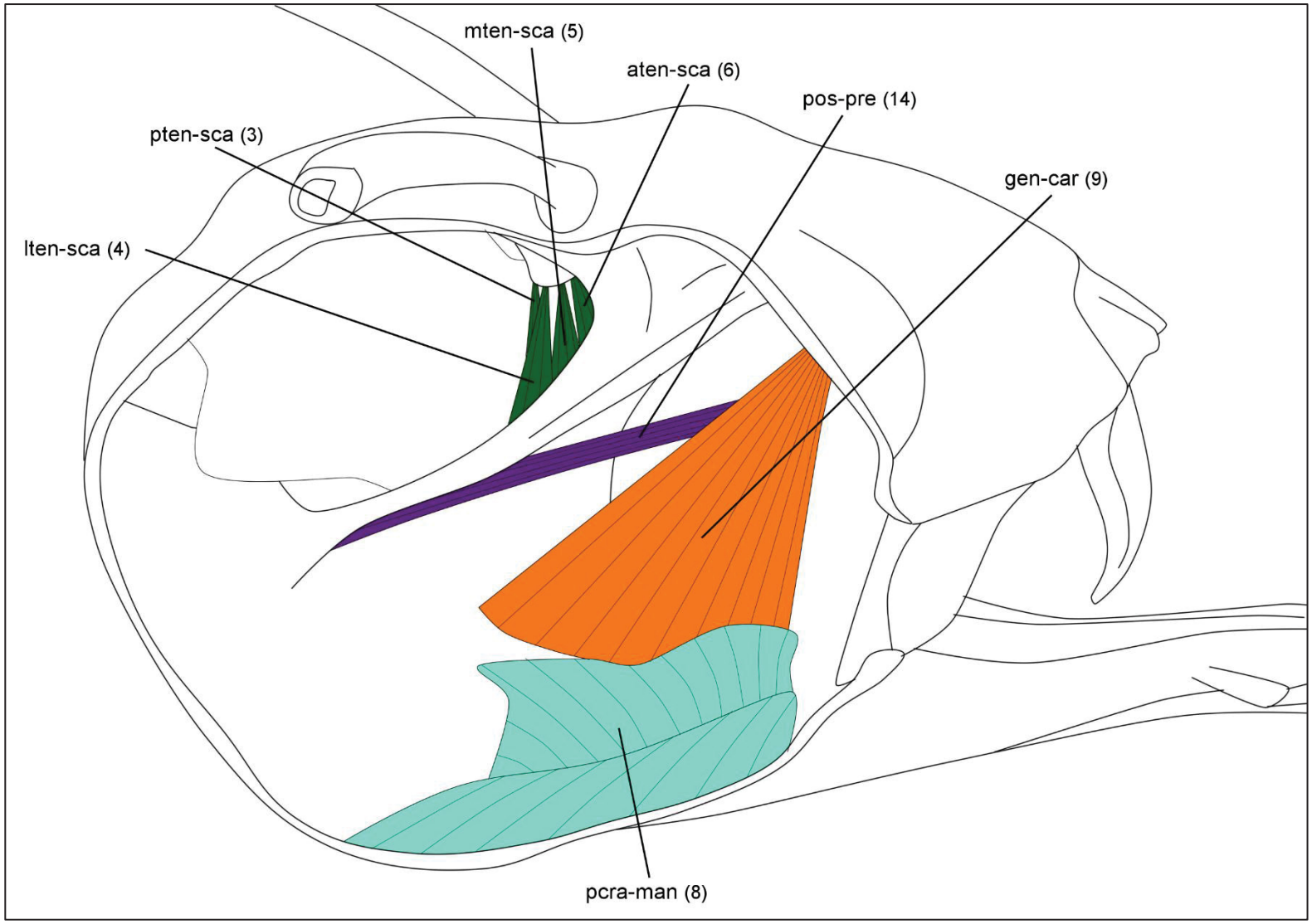

FIGURE 31. EXTRINSIC MUSCLES OF THE HEAD OF MALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

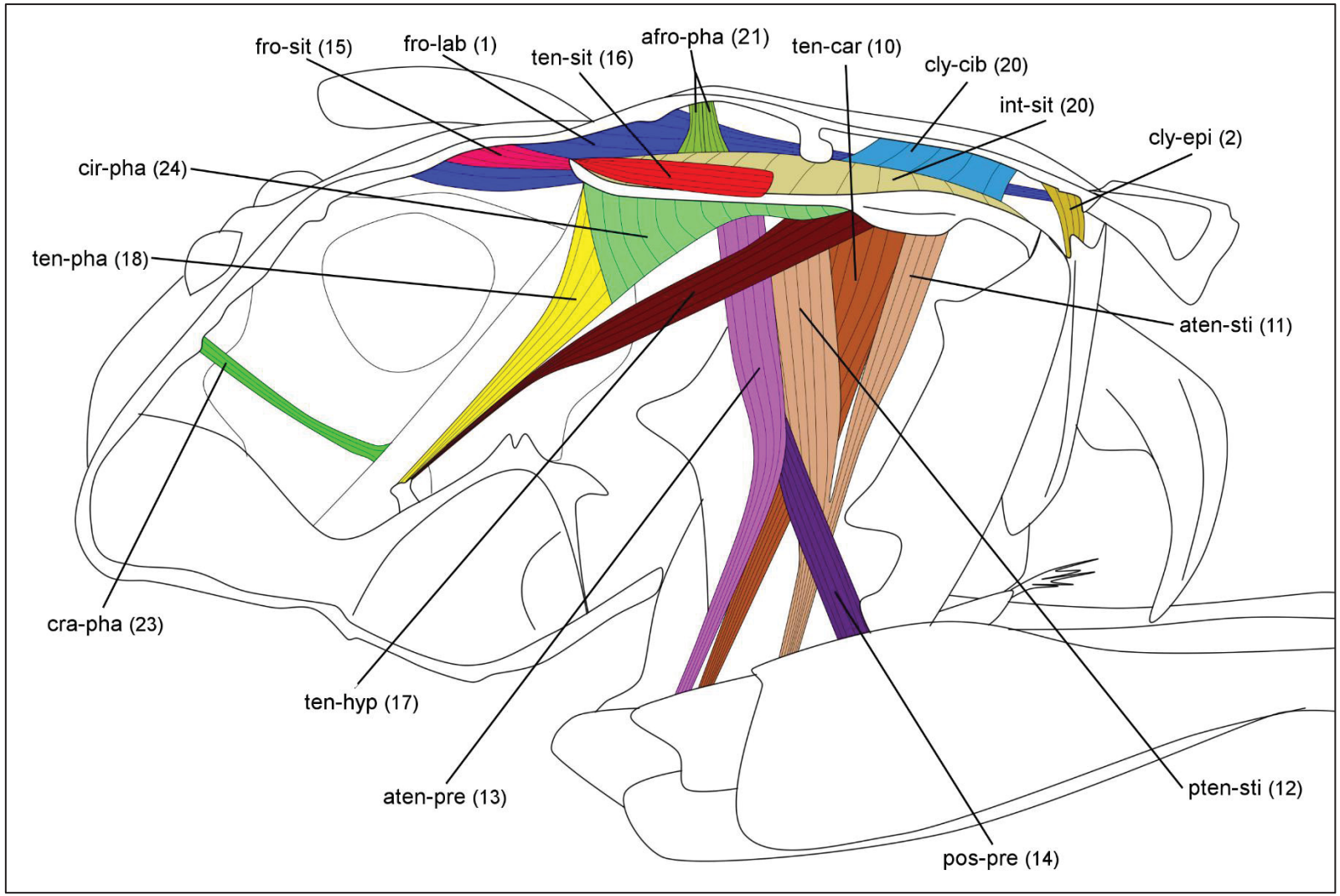

FIGURE 32. EXTRINSIC MUSCLES OF THE HEAD OF MALE *PSAENYTHIA BERGII* HOLMBERG, 1884 (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

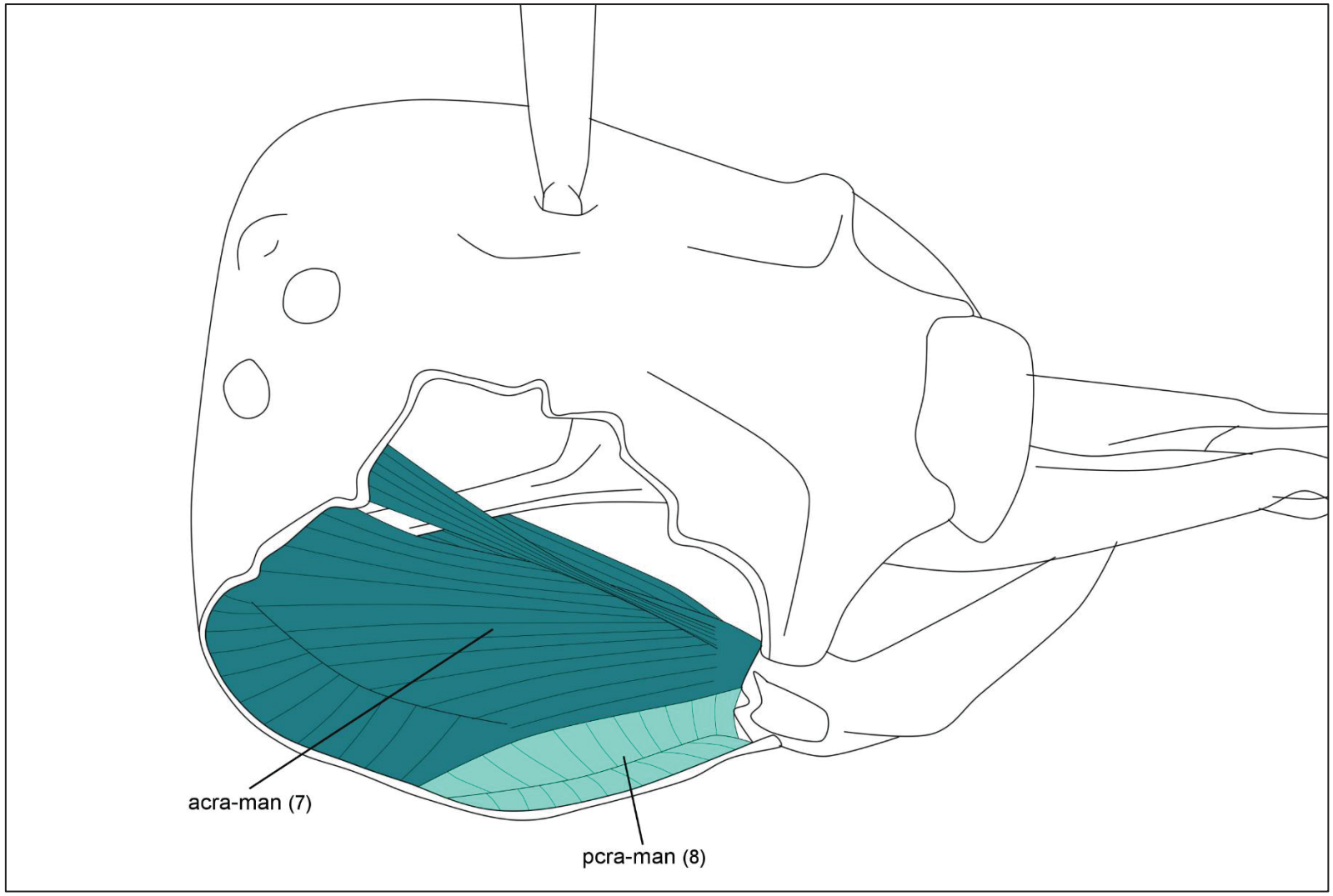

FIGURE 33. EXTRINSIC MUSCLES OF THE HEAD OF MALE *ANTHRENOIDES MERIDIONALIS* (SCHROTTKY, 1906) (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

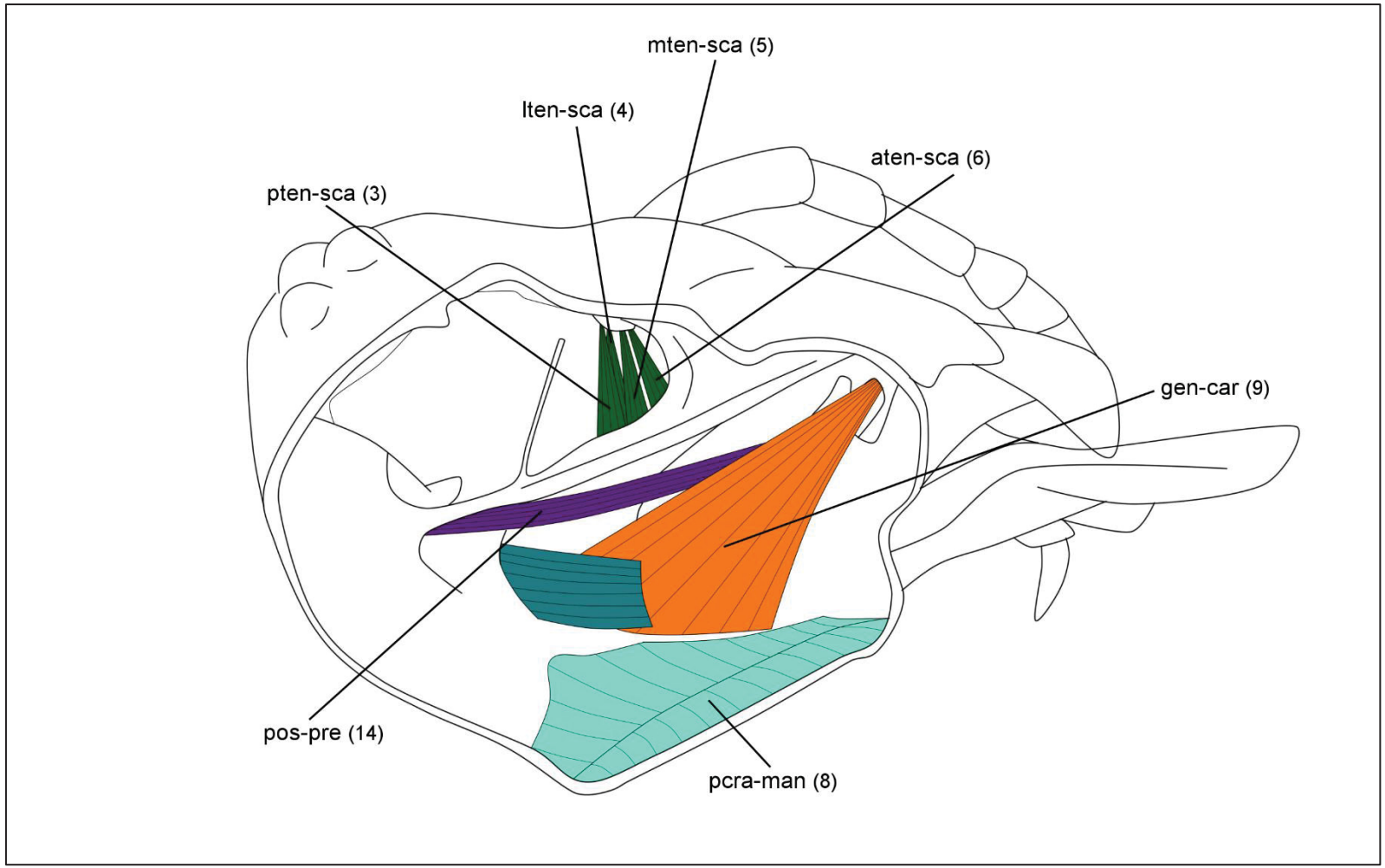

FIGURE 34. EXTRINSIC MUSCLES OF THE HEAD OF MALE *ANTHRENOIDES MERIDIONALIS* (SCHROTTKY, 1906) (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

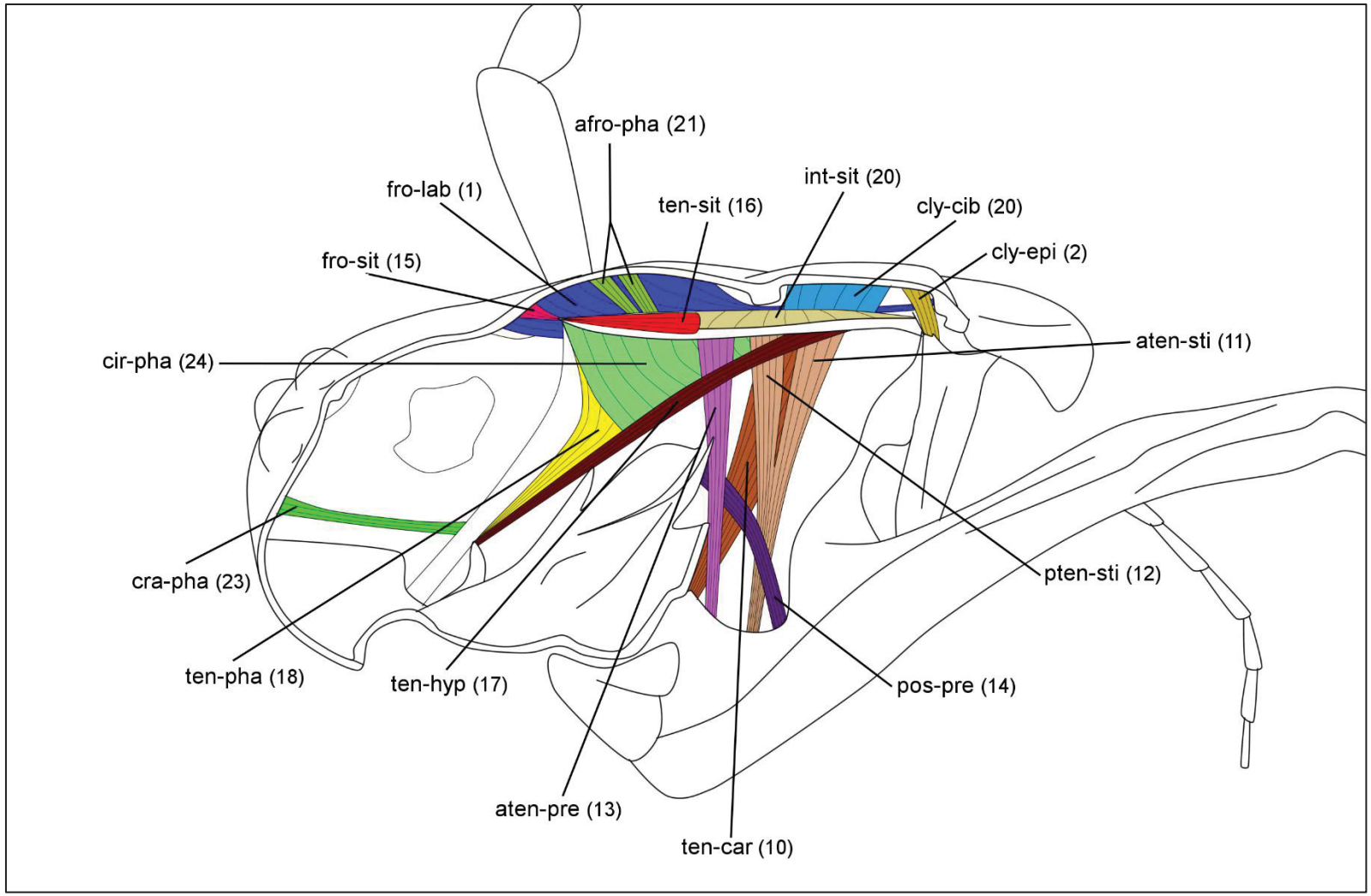

FIGURE 35. EXTRINSIC MUSCLES OF THE HEAD OF MALE *ANTHRENOIDES MERIDIONALIS* (SCHROTTKY, 1906) (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

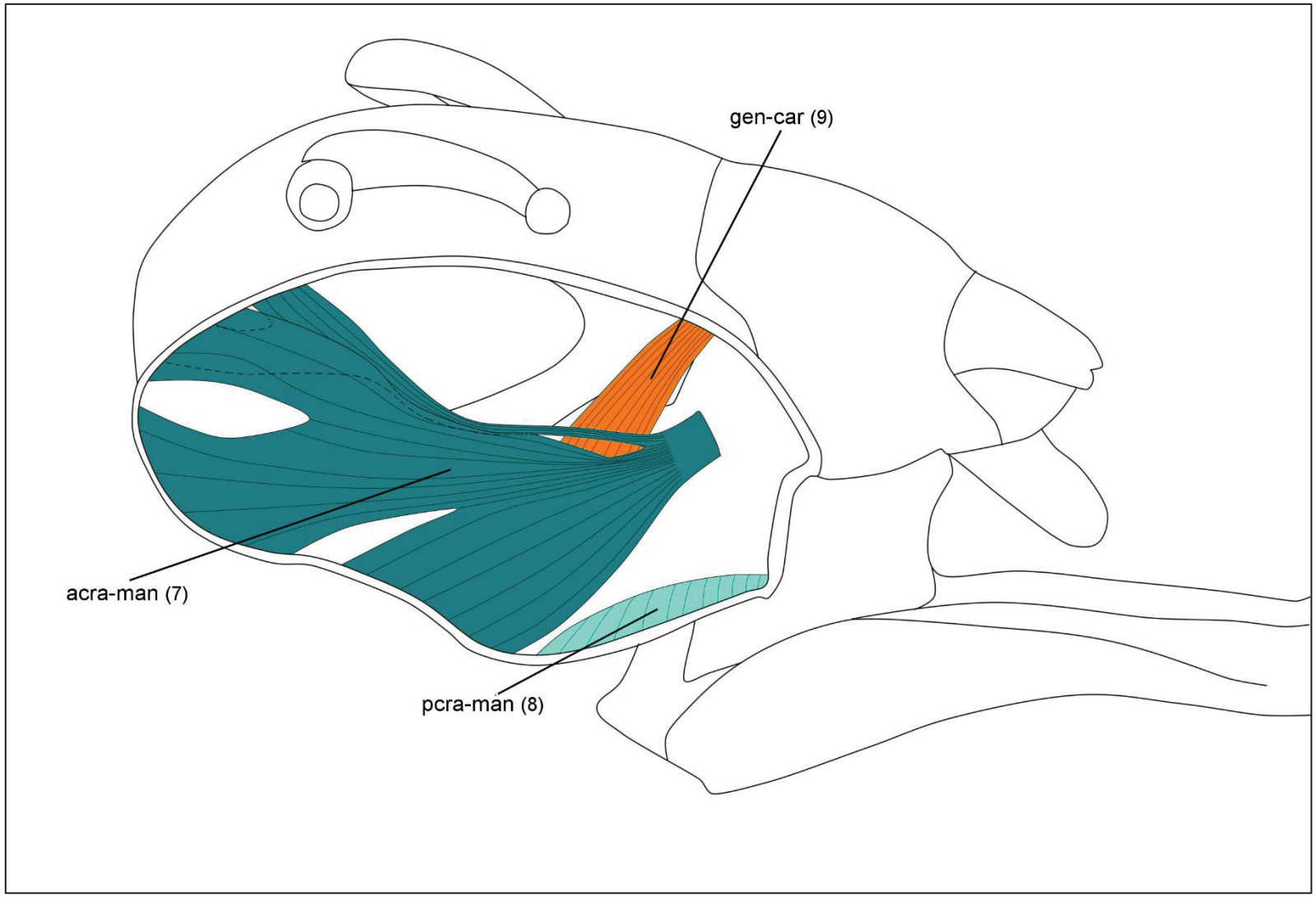

FIGURE 36. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ROPHITULUS* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

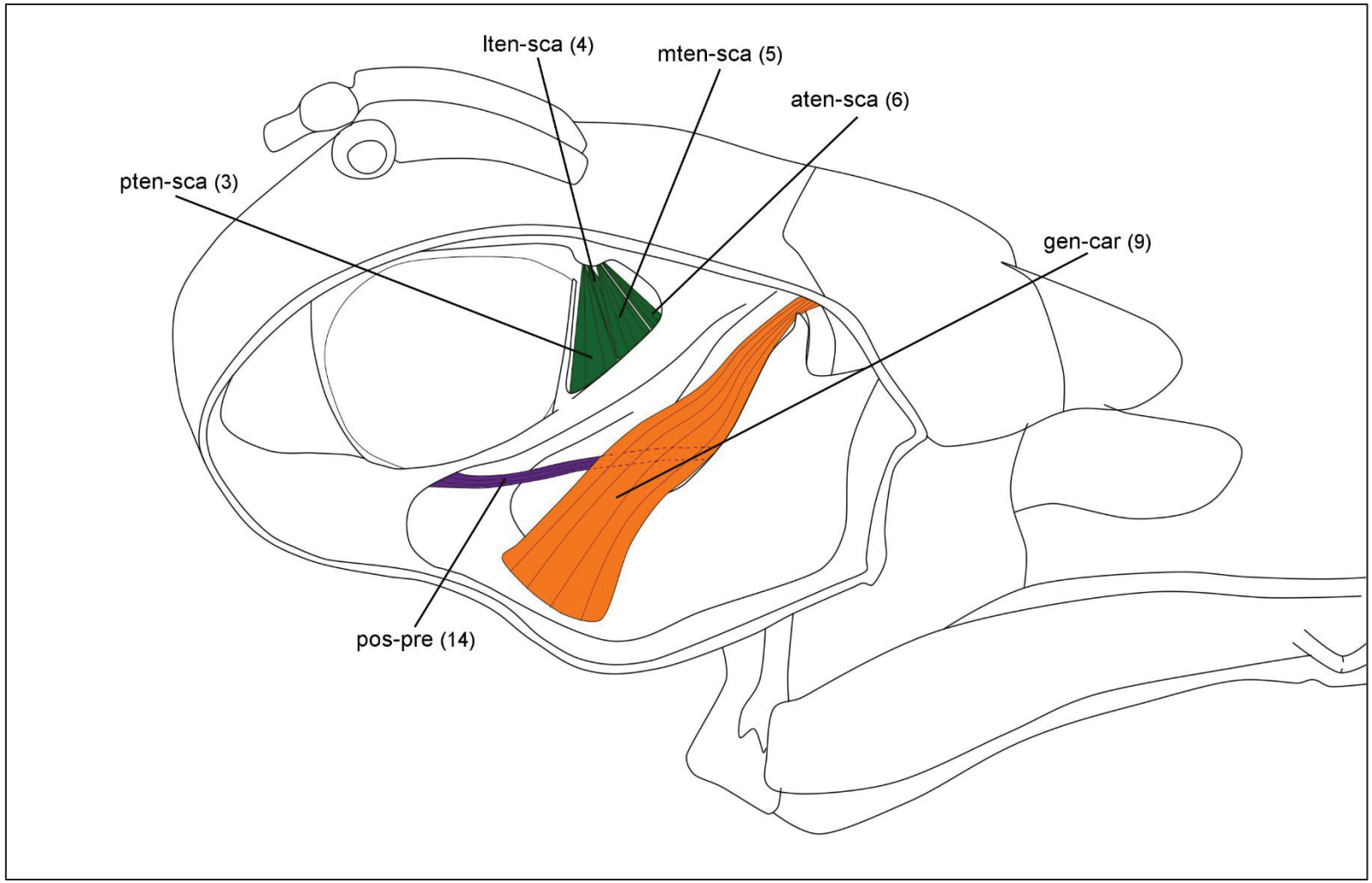

FIGURE 37. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ROPHITULUS* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

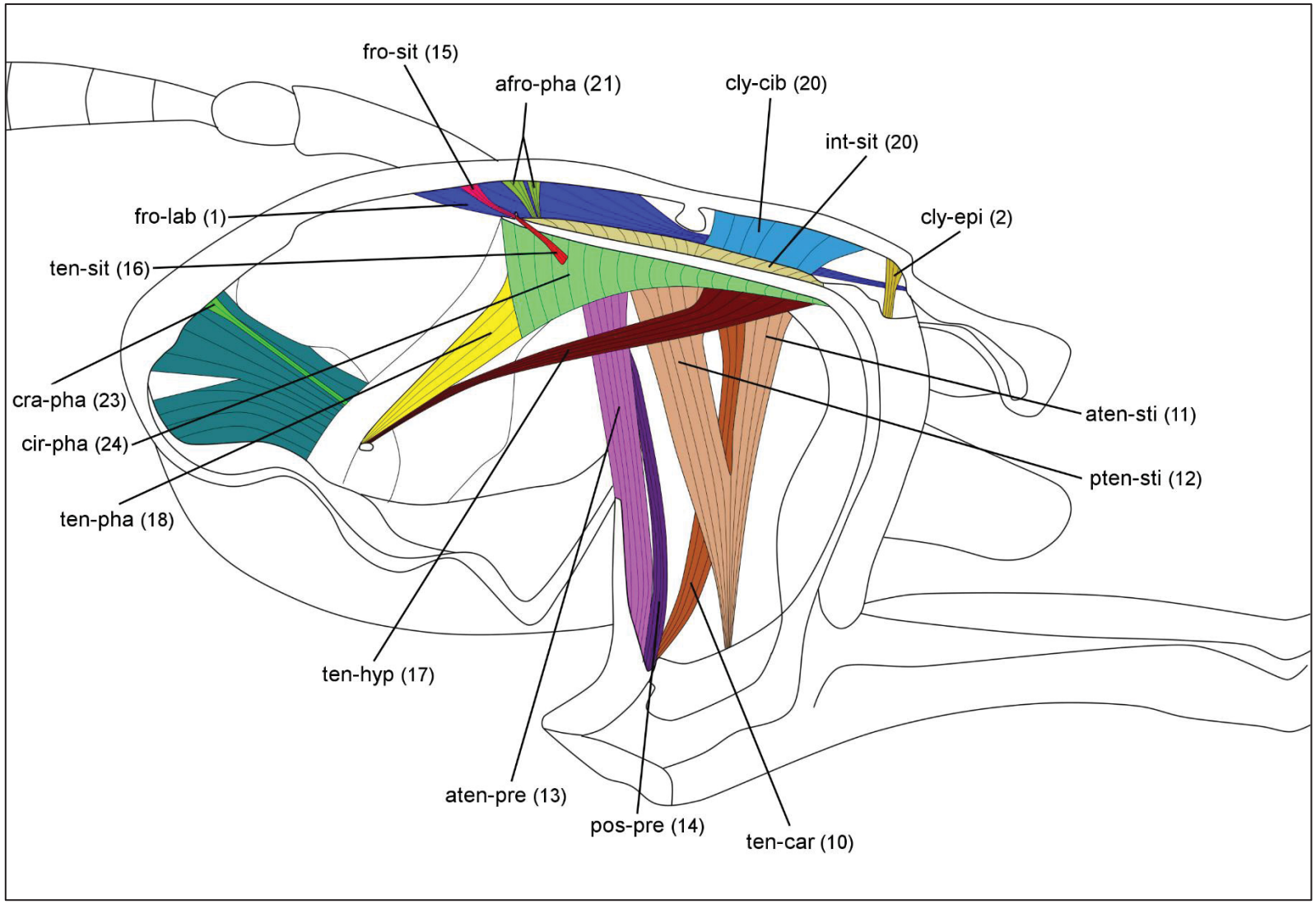

FIGURE 38. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ROPHITULUS* SP. (APIDAE: ANDRENINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

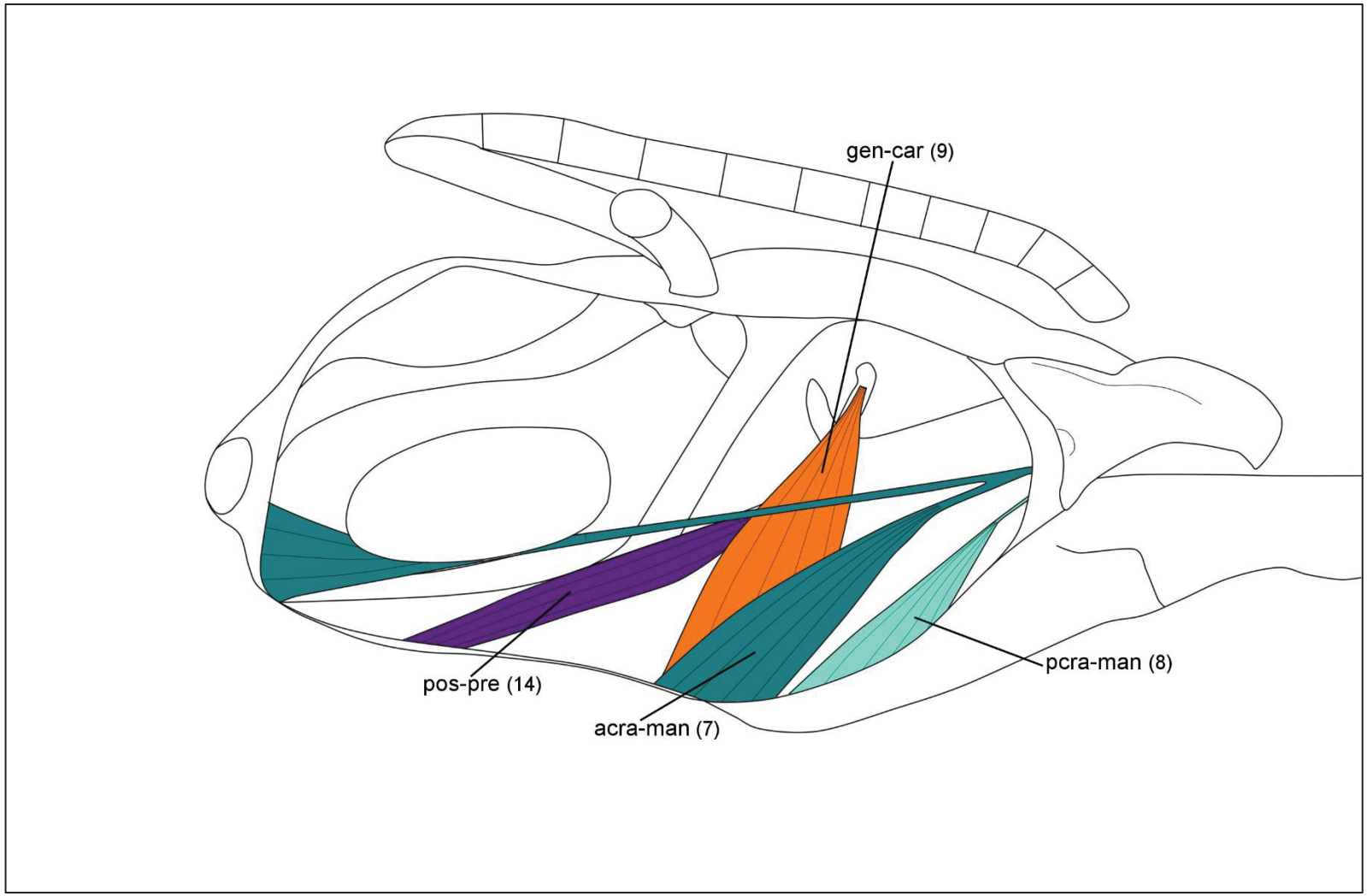

FIGURE 39. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *APIS MELLIFERA* LINNAEUS, 1758 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

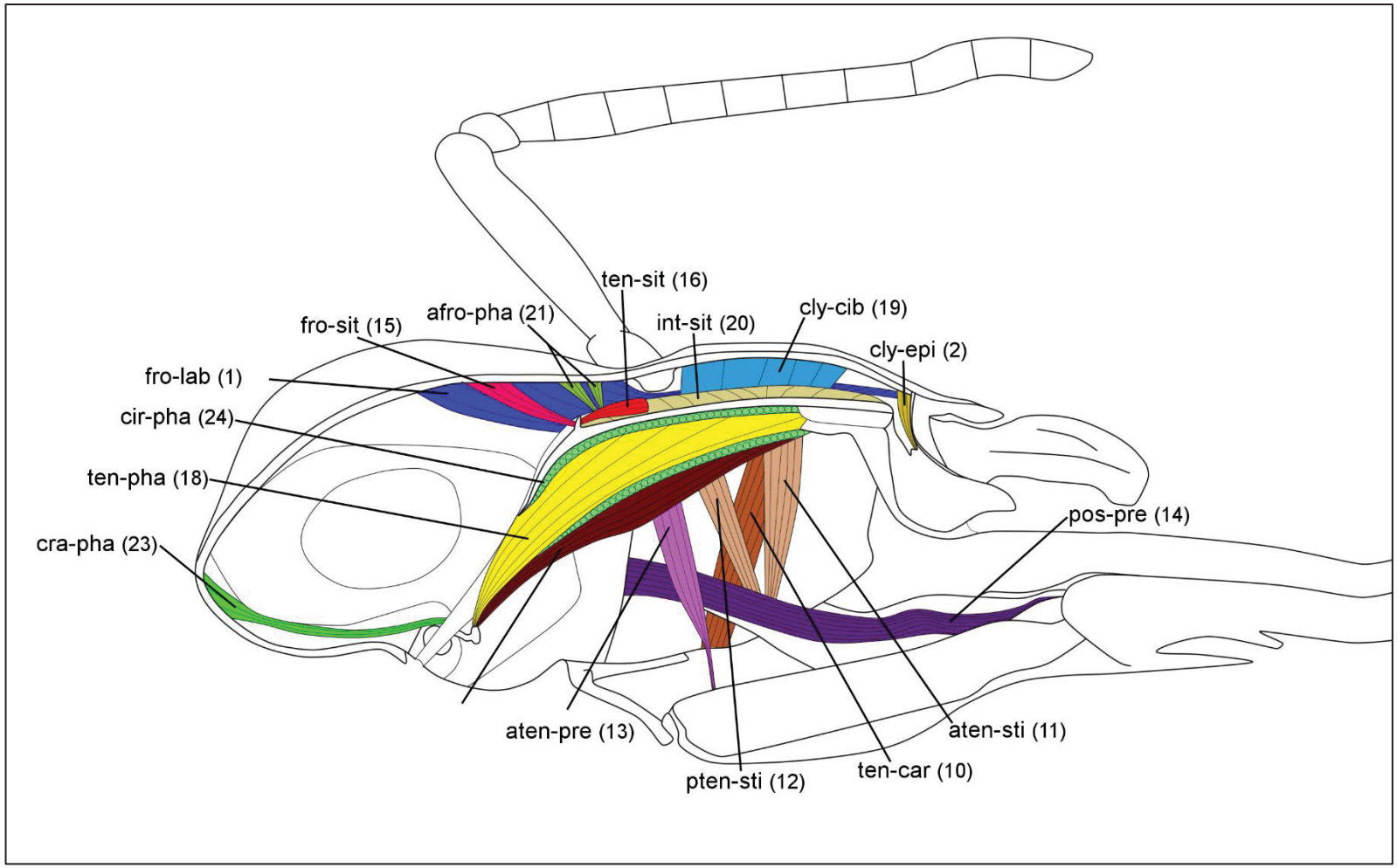

FIGURE 40. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *APIS MELLIFERA* LINNAEUS, 1758 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

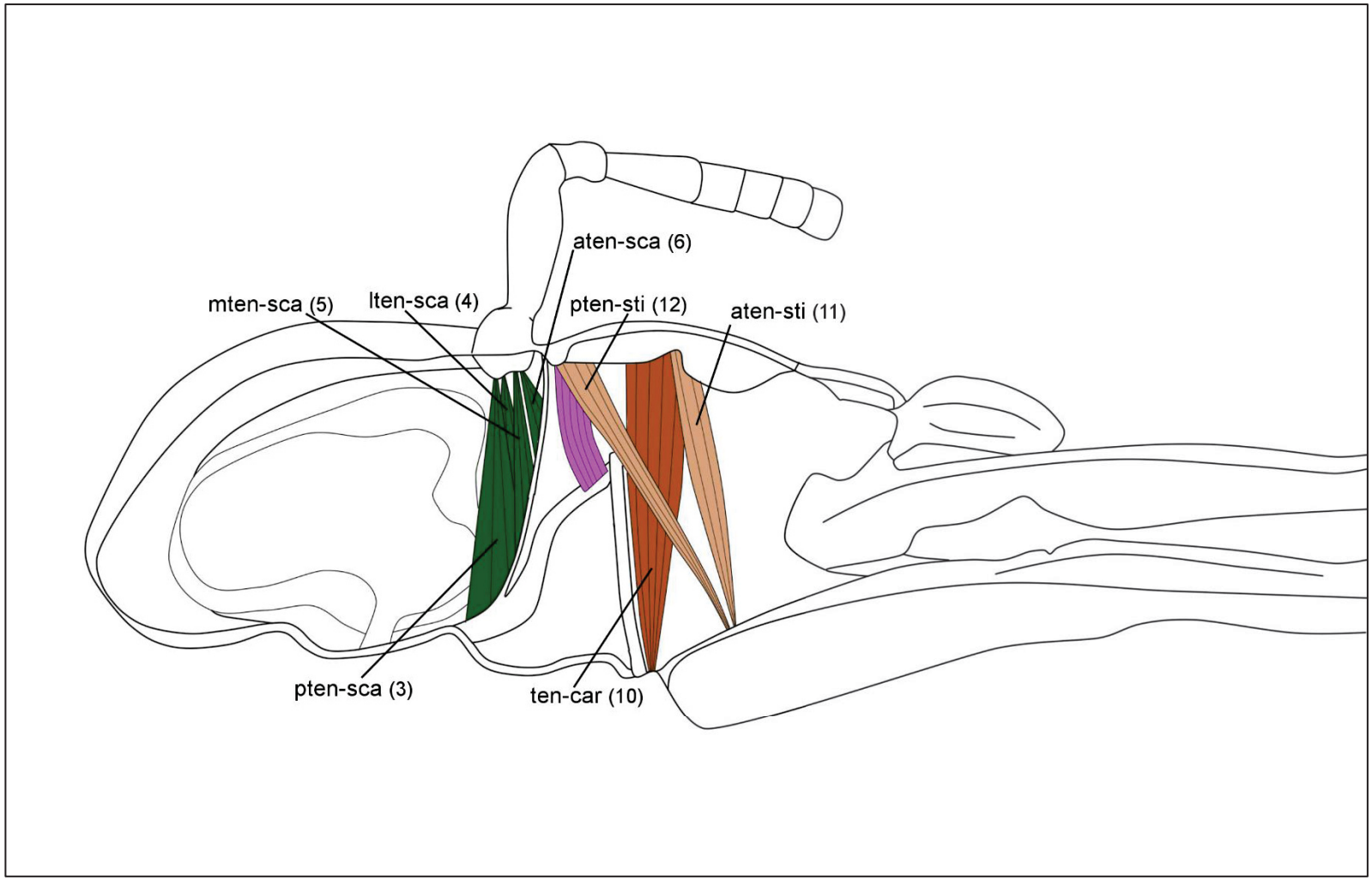

FIGURE 41. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *APIS MELLIFERA* LINNAEUS, 1758 (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

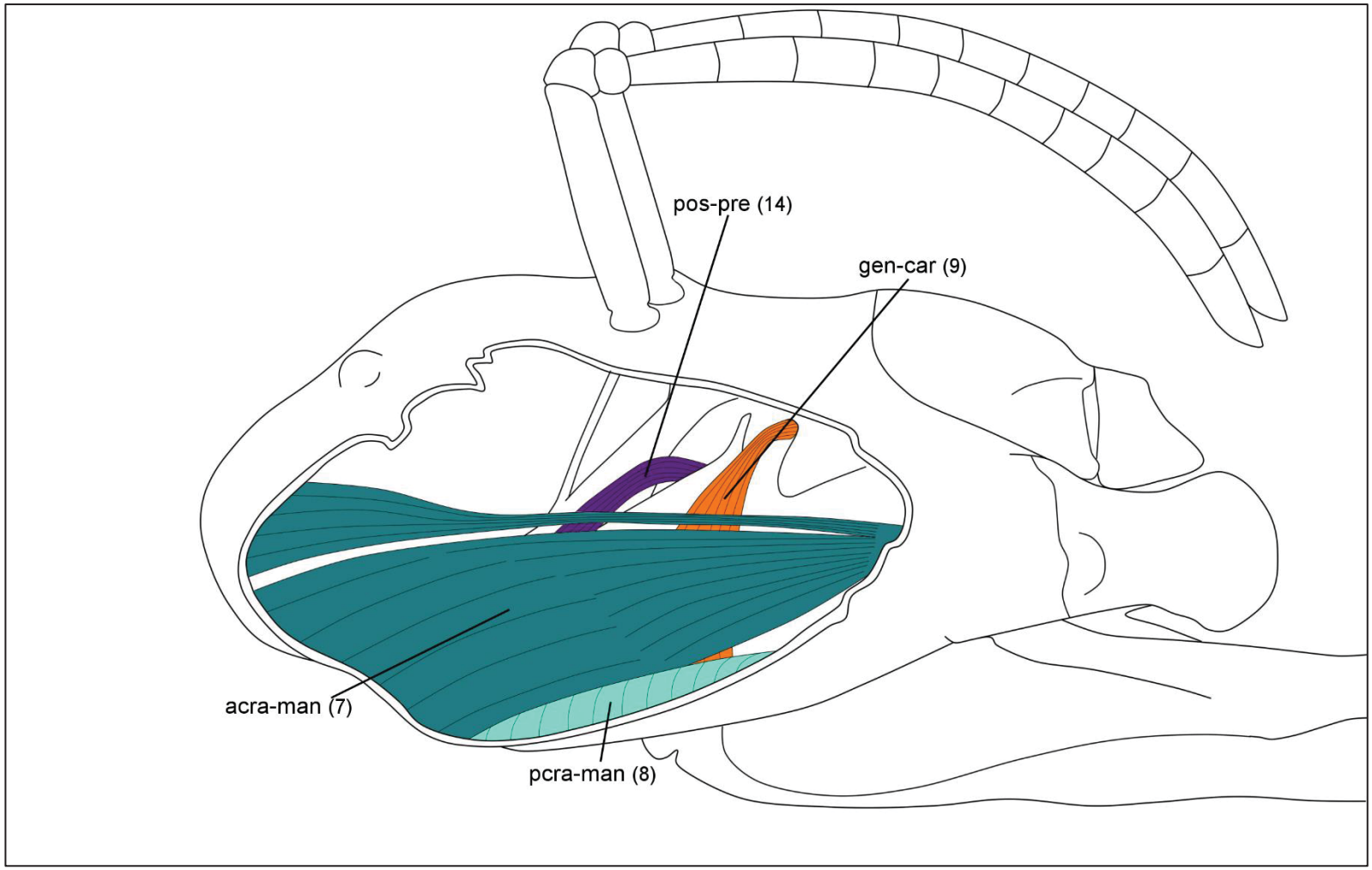

FIGURE 42. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *BOMBUS (FERVIDOBOMBUS) MORIO* (SWEDERUS, 1787) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

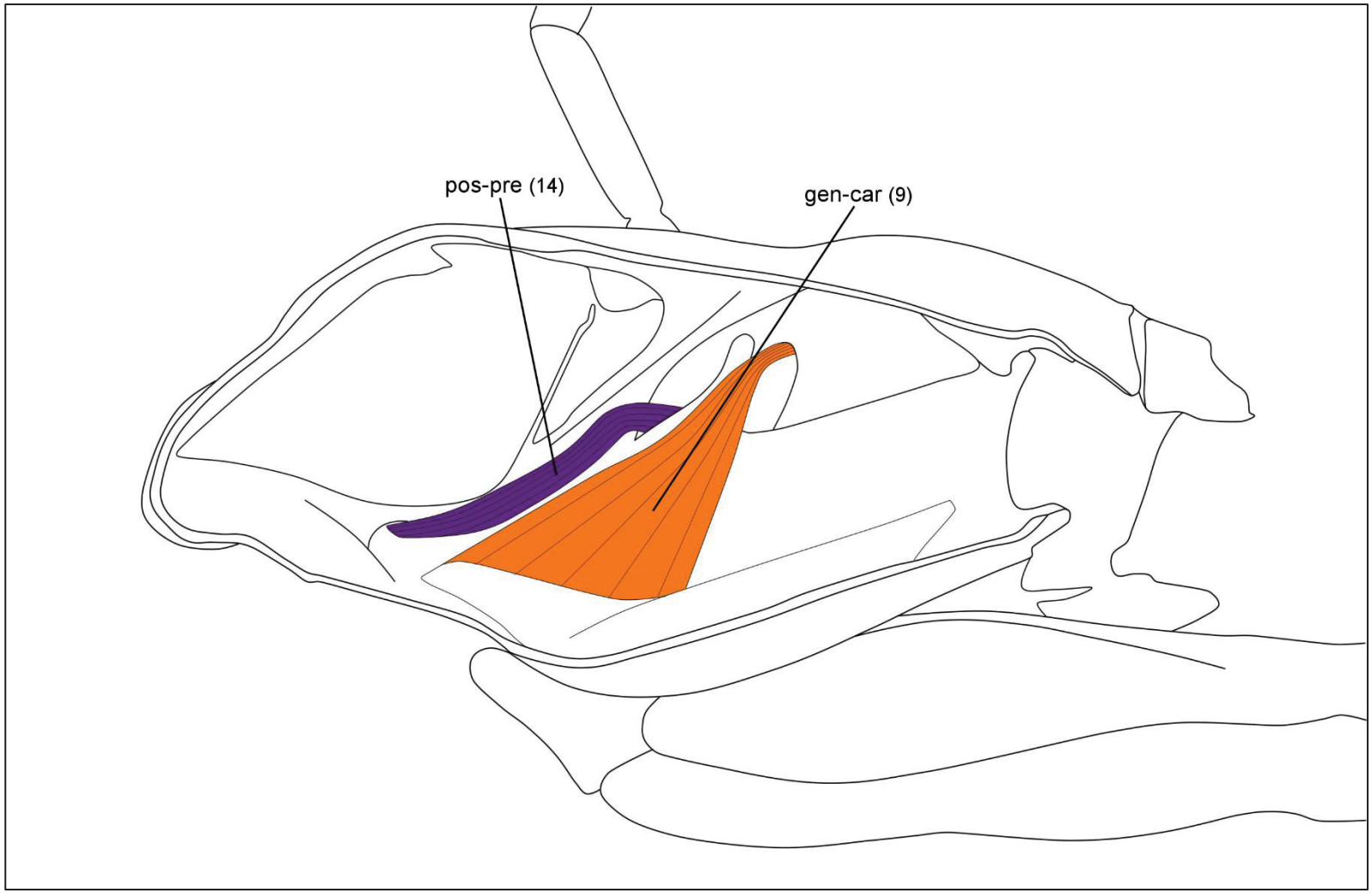

FIGURE 43. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *BOMBUS (FERVIDOBOMBUS) MORIO* (SWEDERUS, 1787) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

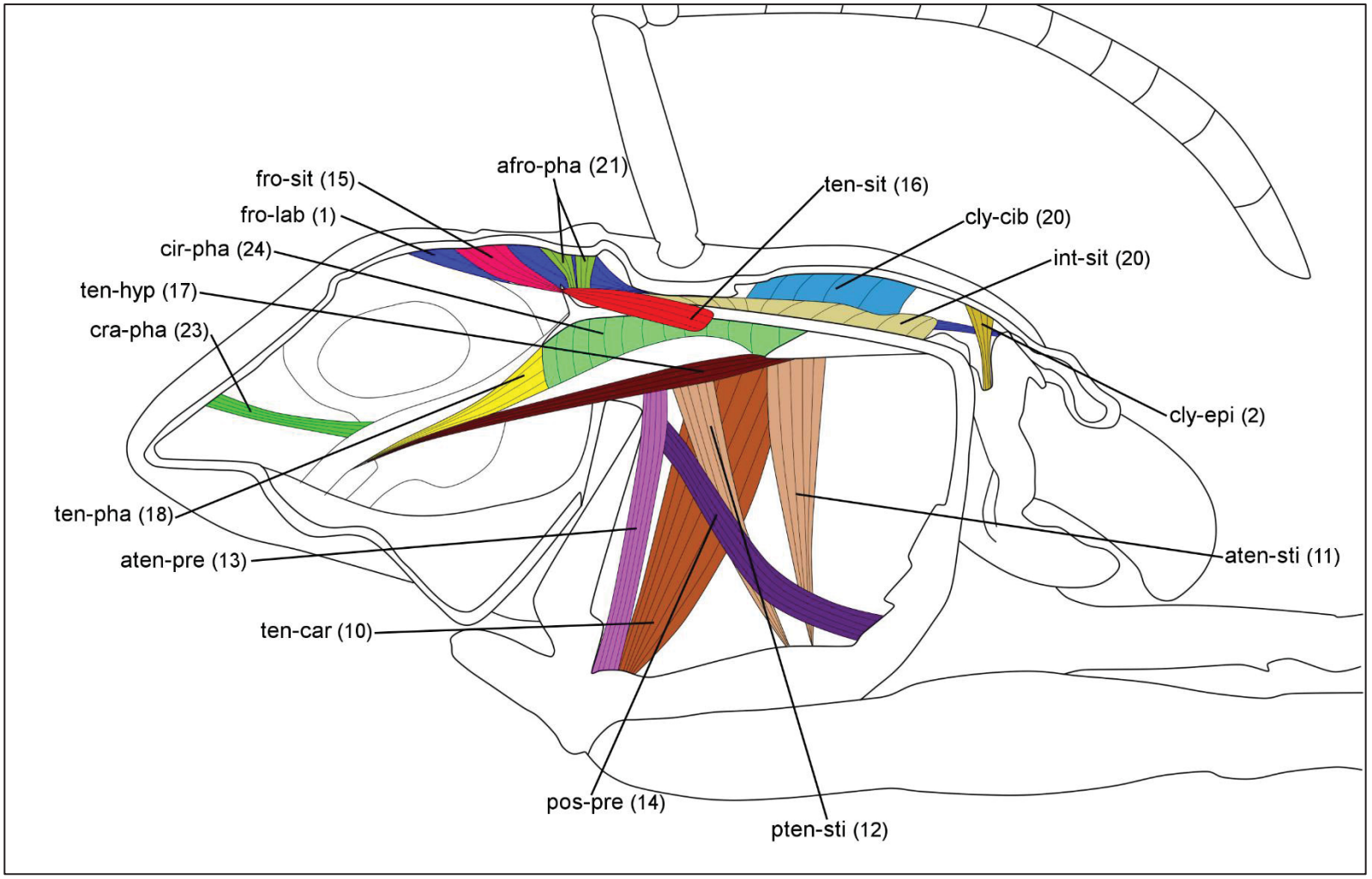

FIGURE 44. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *BOMBUS (FERVIDOBOMBUS) MORIO* (SWEDERUS, 1787) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

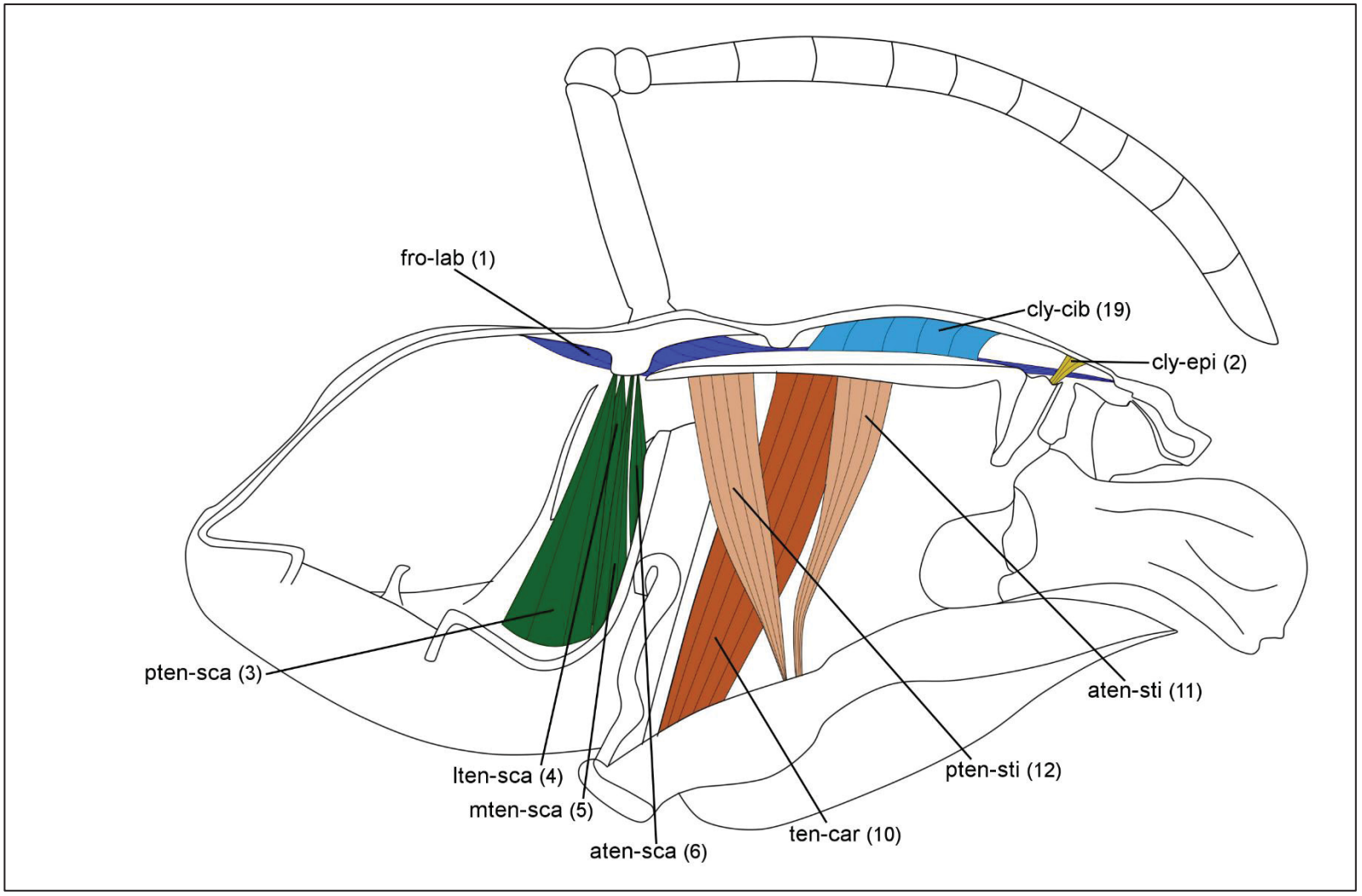

FIGURE 45. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *BOMBUS (FERVIDOBOMBUS) MORIO* (SWEDERUS, 1787) (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

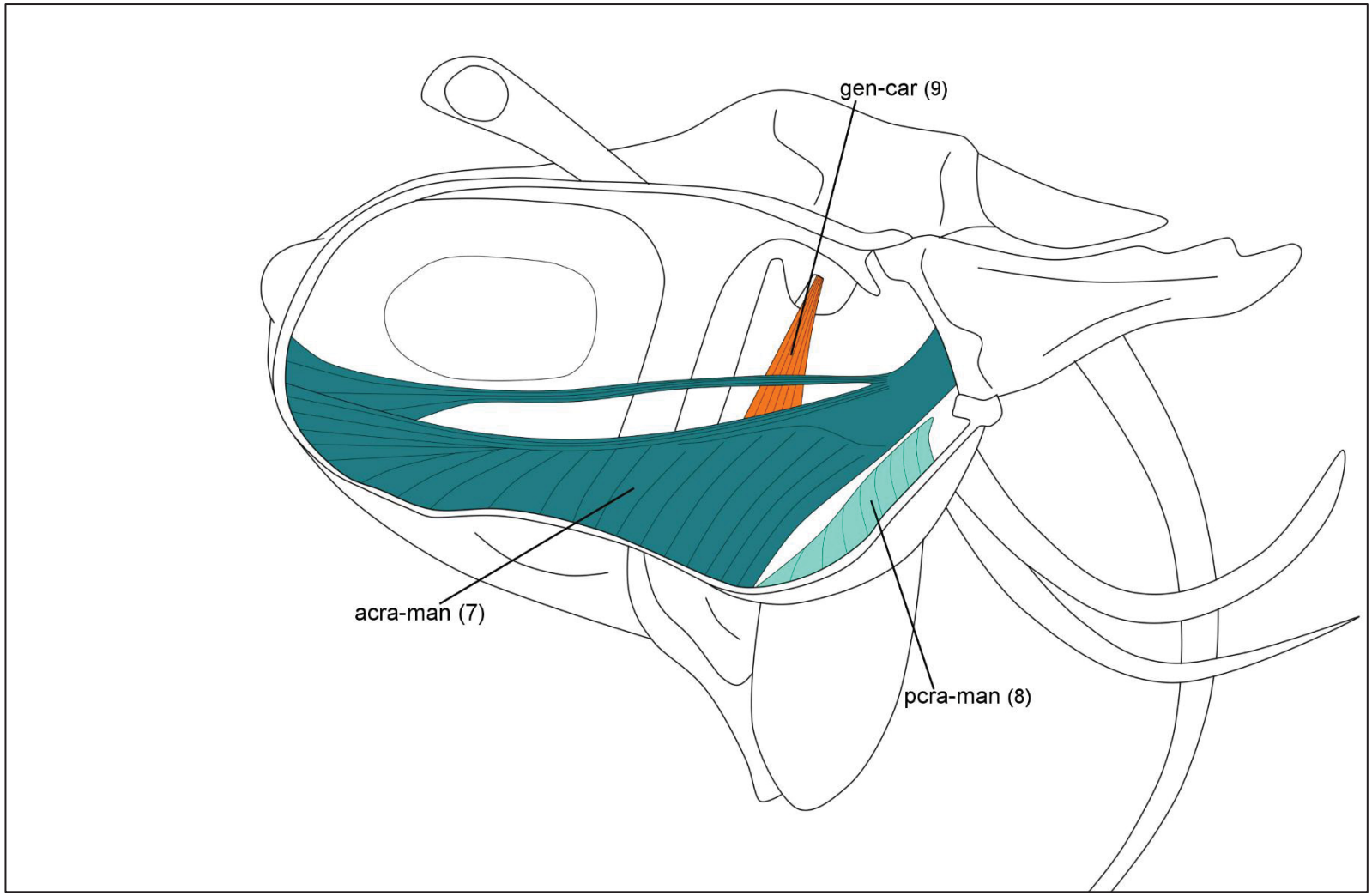

FIGURE 46. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CENTRIS (HEMISIELLA) TARSATA* SMITH, 1874 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

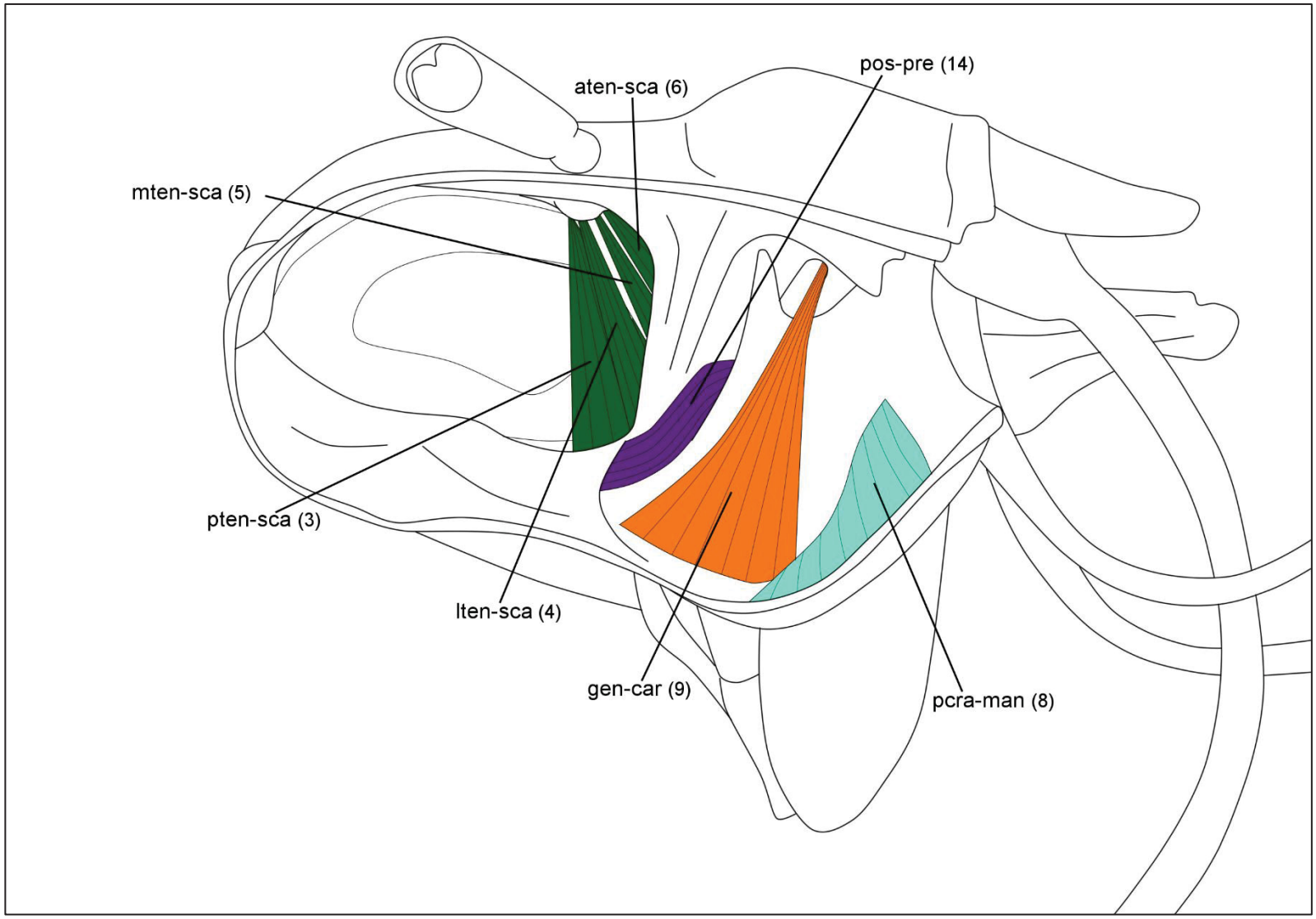

FIGURE 47. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CENTRIS (HEMISIELLA) TARSATA* SMITH, 1874 (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

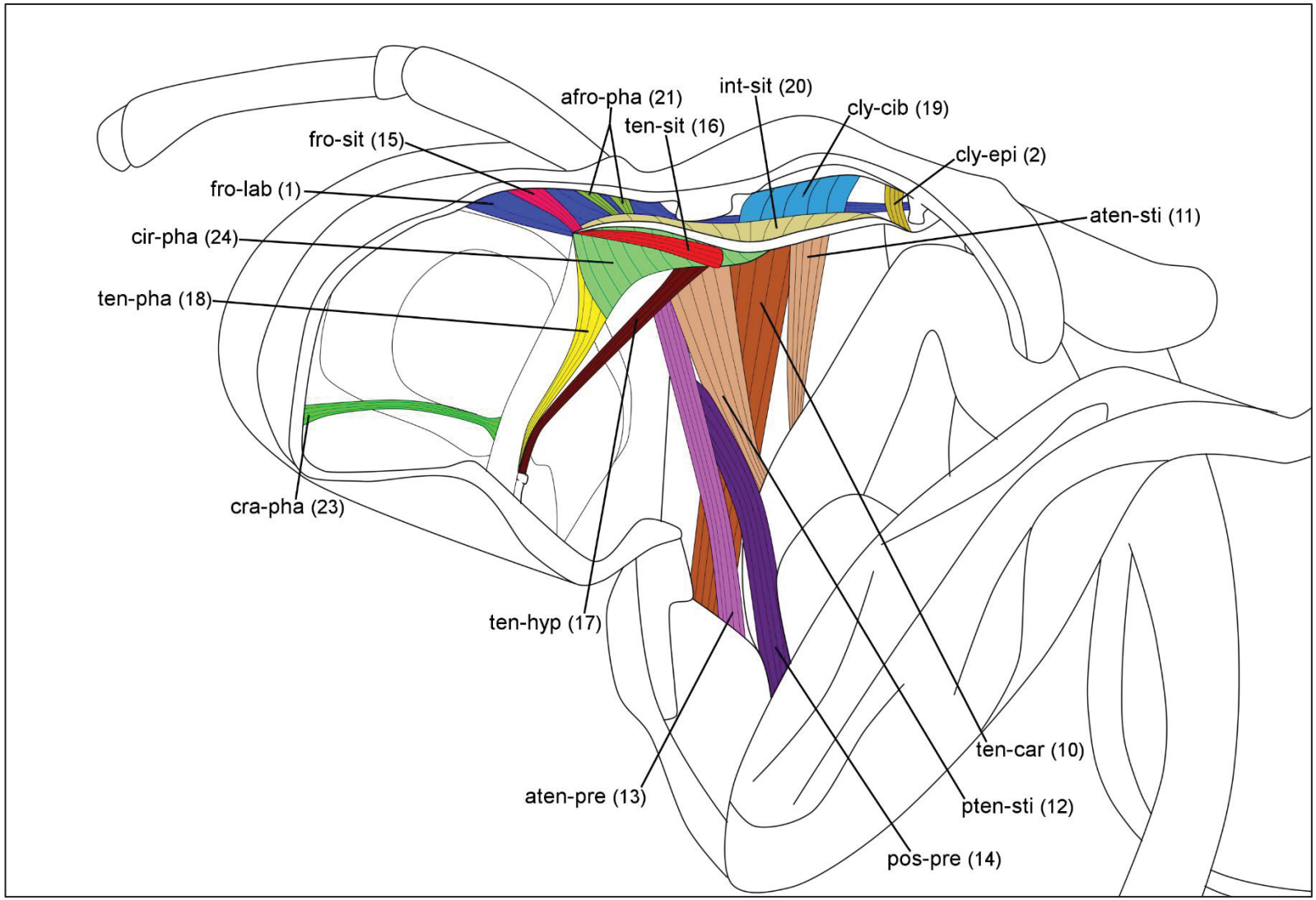

FIGURE 48. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CENTRIS (HEMISIELLA) TARSATA* SMITH, 1874 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

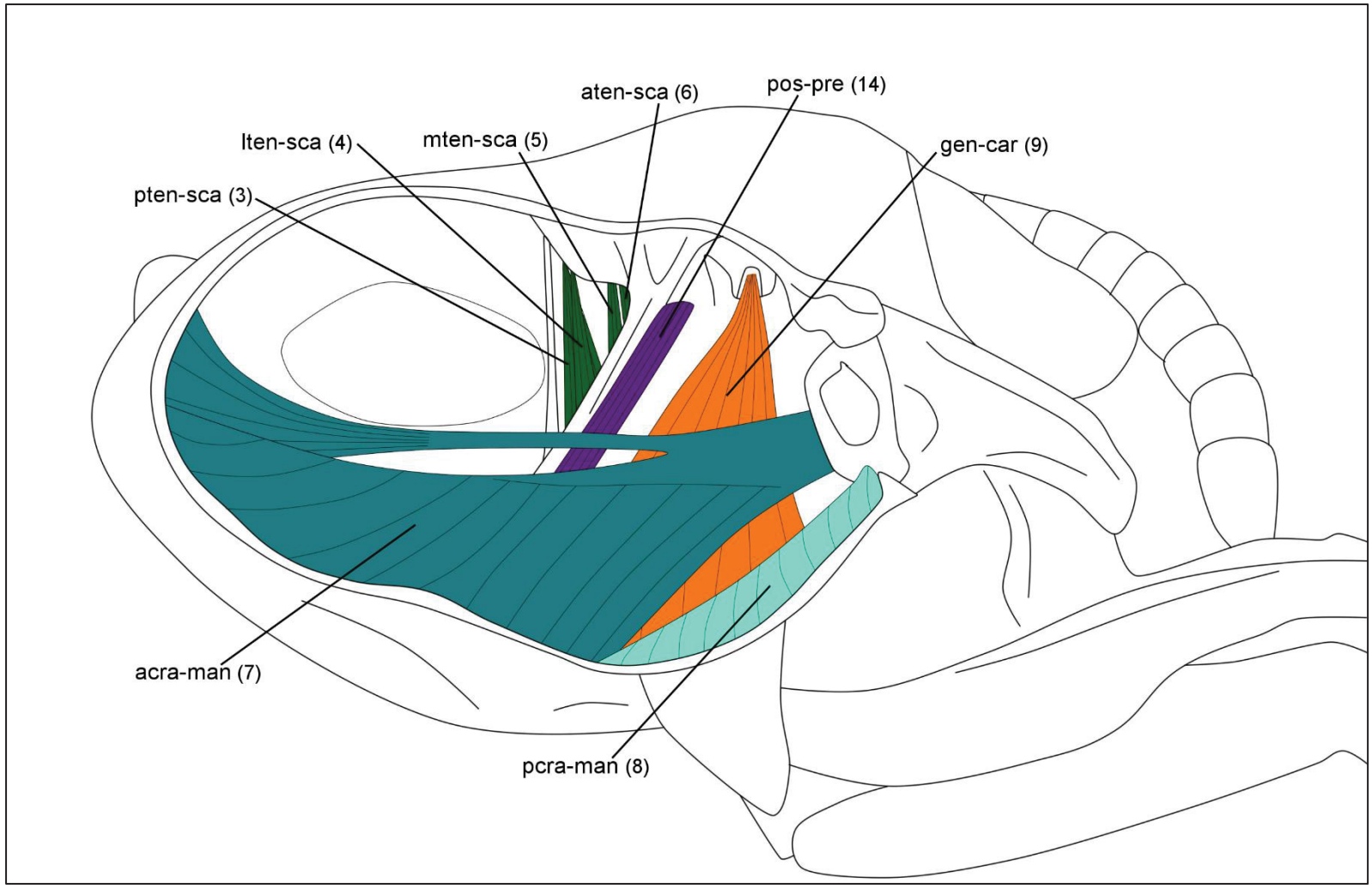

FIGURE 49. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITOMA SEGMENTARIA* (FABRICIUS 1804) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

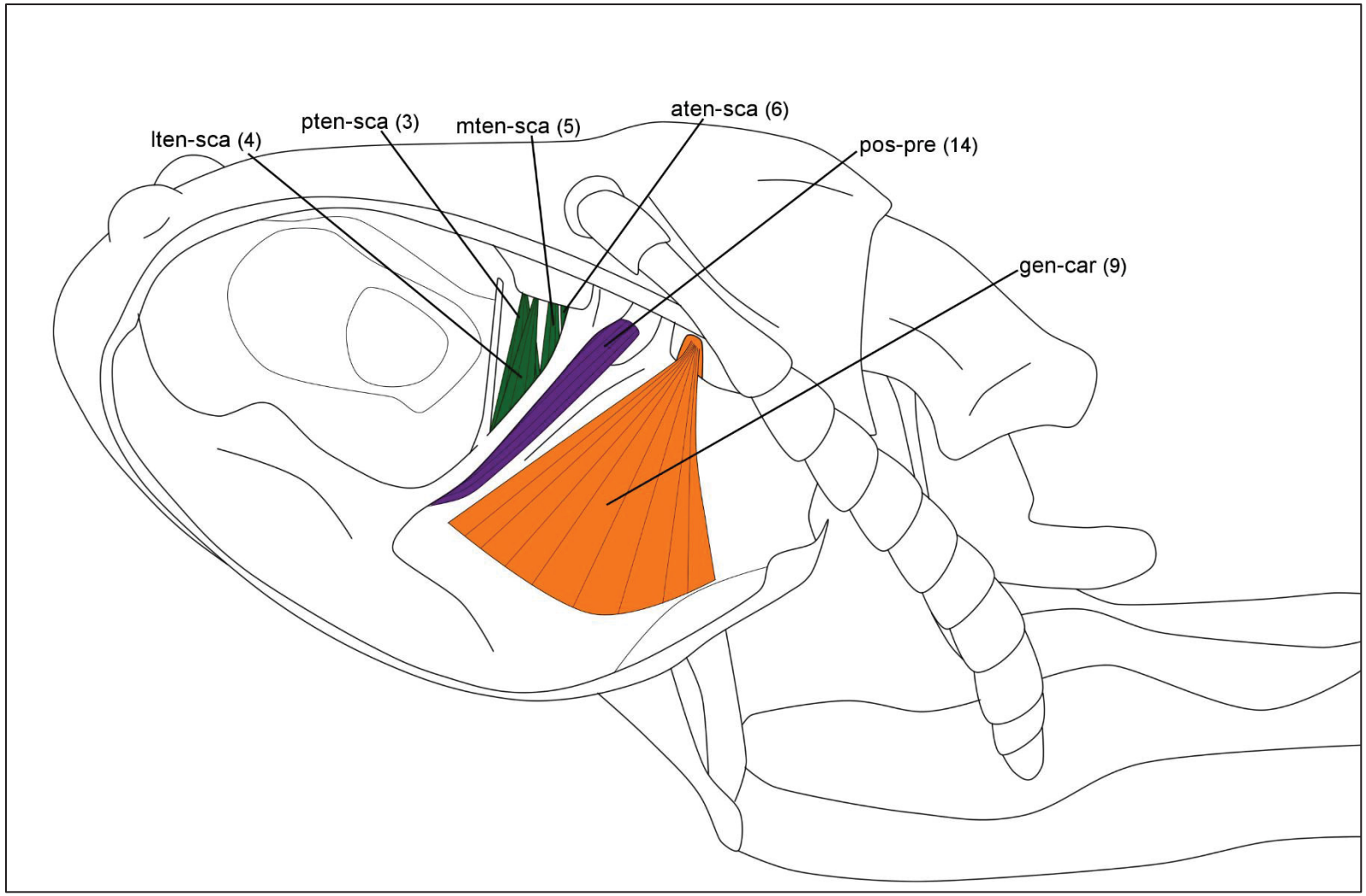

FIGURE 50. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITOMA SEGMENTARIA* (FABRICIUS 1804) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

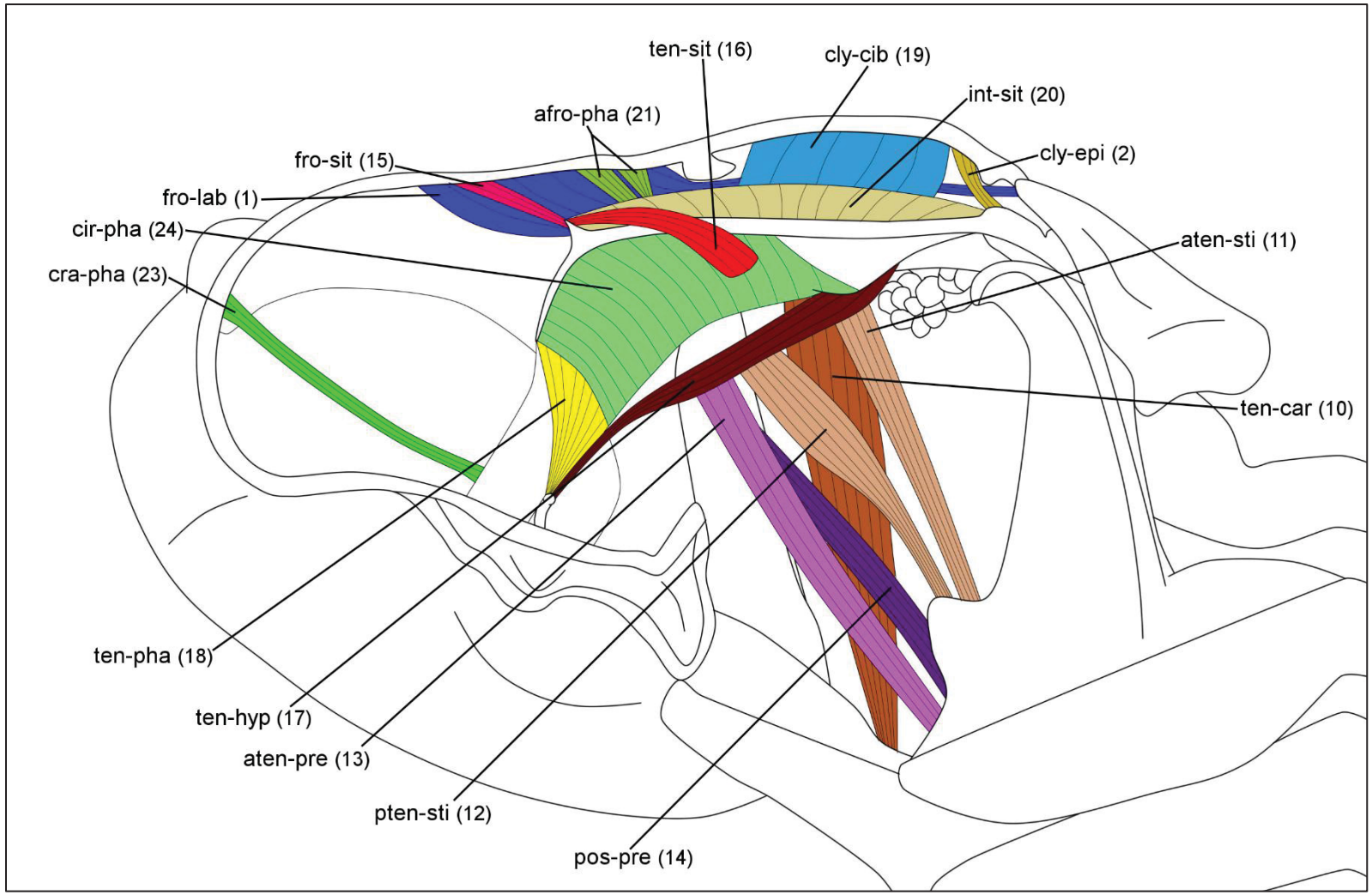

FIGURE 51. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITOMA SEGMENTARIA* (FABRICIUS 1804) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

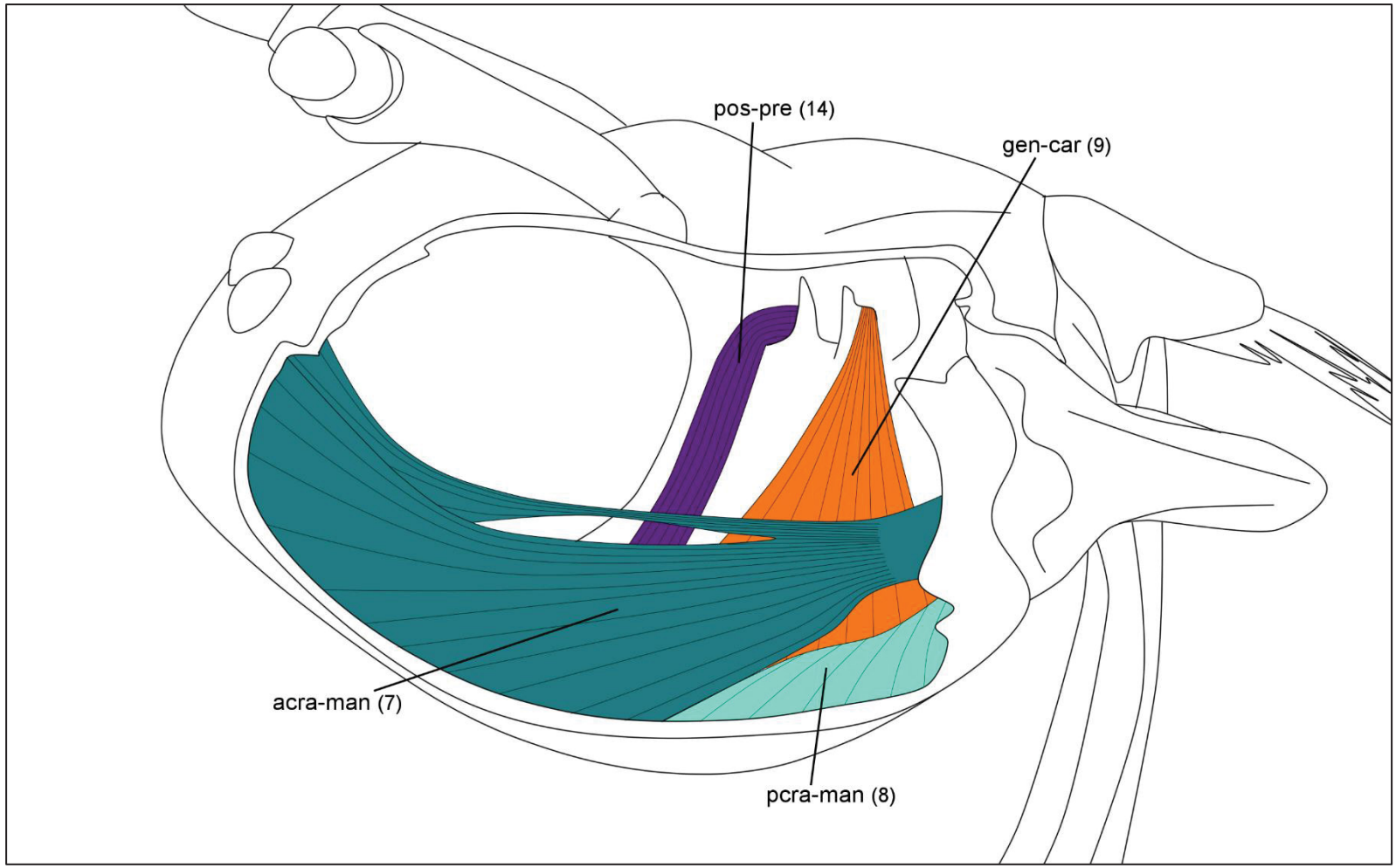

FIGURE 52. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MESOPLIA* SP. (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

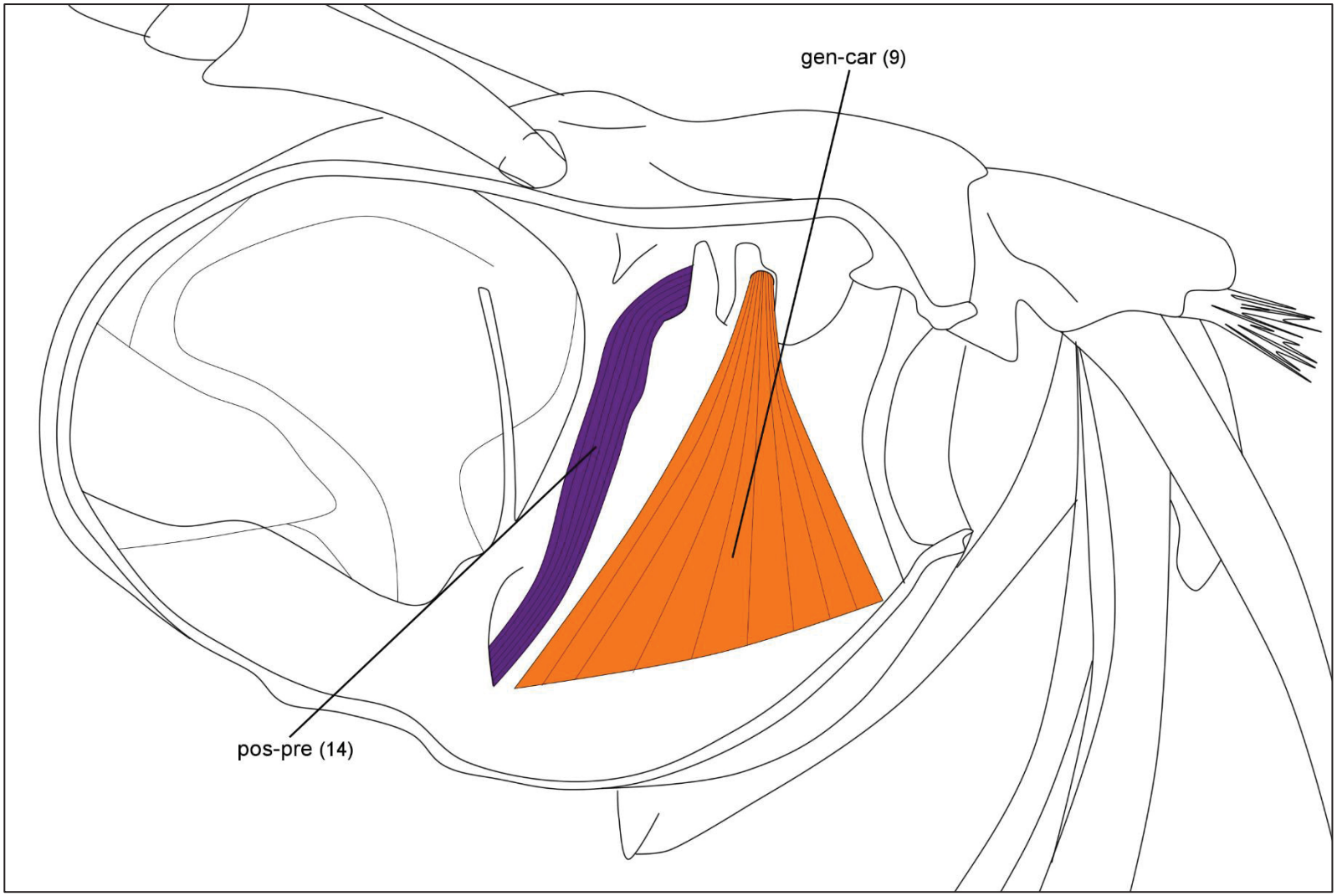

FIGURE 53. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MESOPLIA* SP. (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

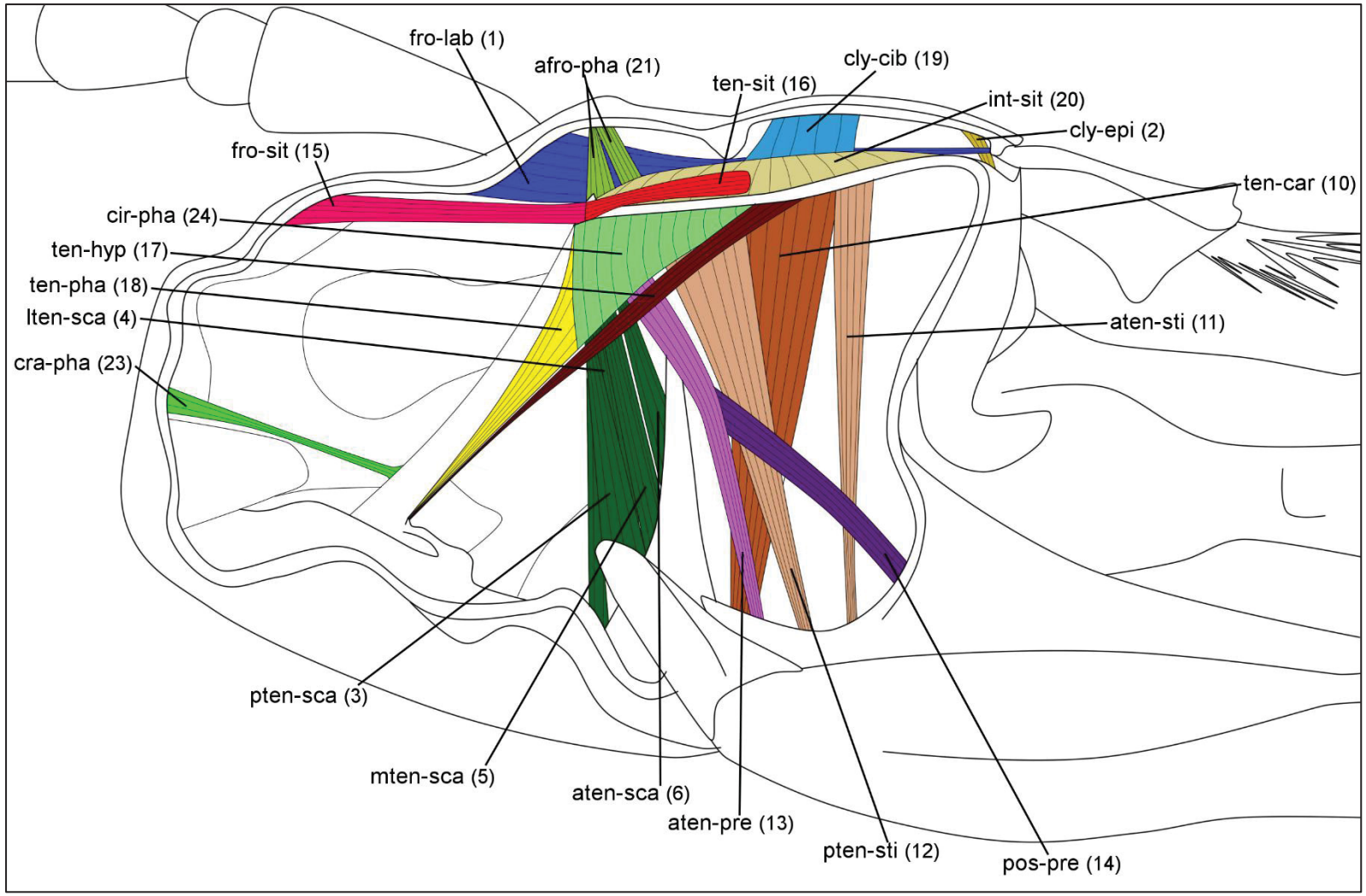

FIGURE 54. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MESOPLIA* SP. (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

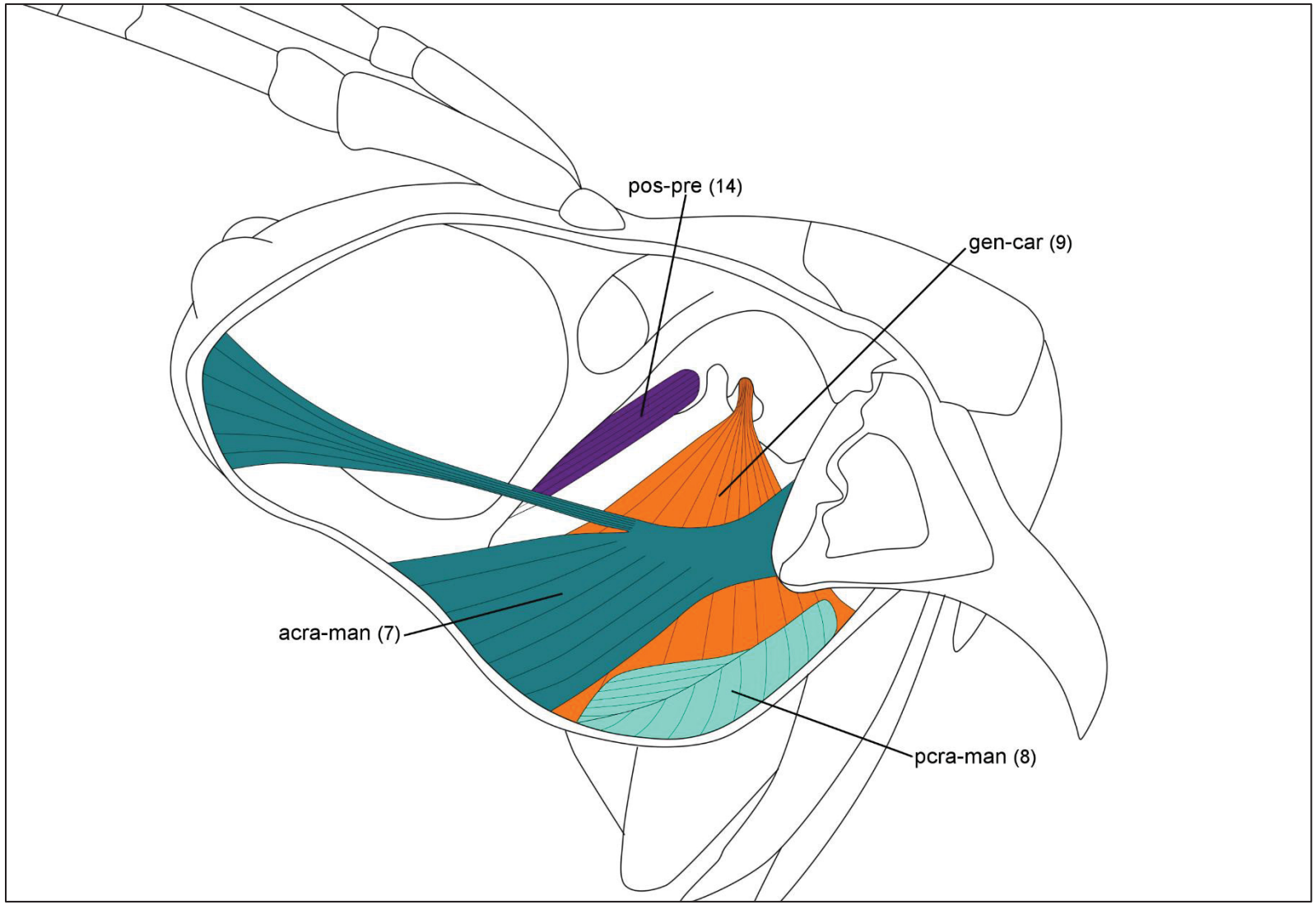

FIGURE 55. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELISSOPTILA AUREOCINCTA* URBAN, 1968 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

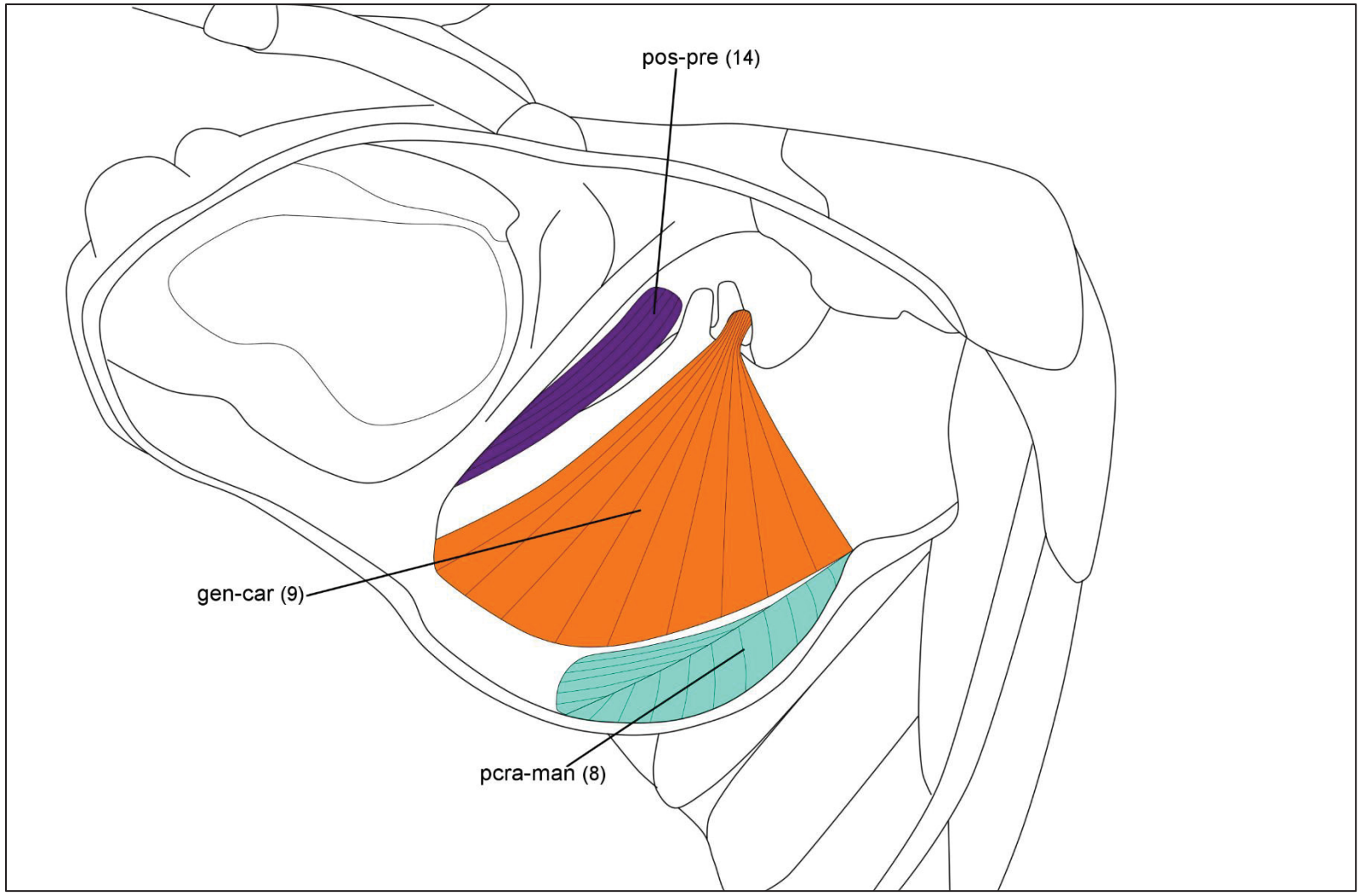

FIGURE 56. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELISSOPTILA AUREOCINCTA* URBAN, 1968 (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

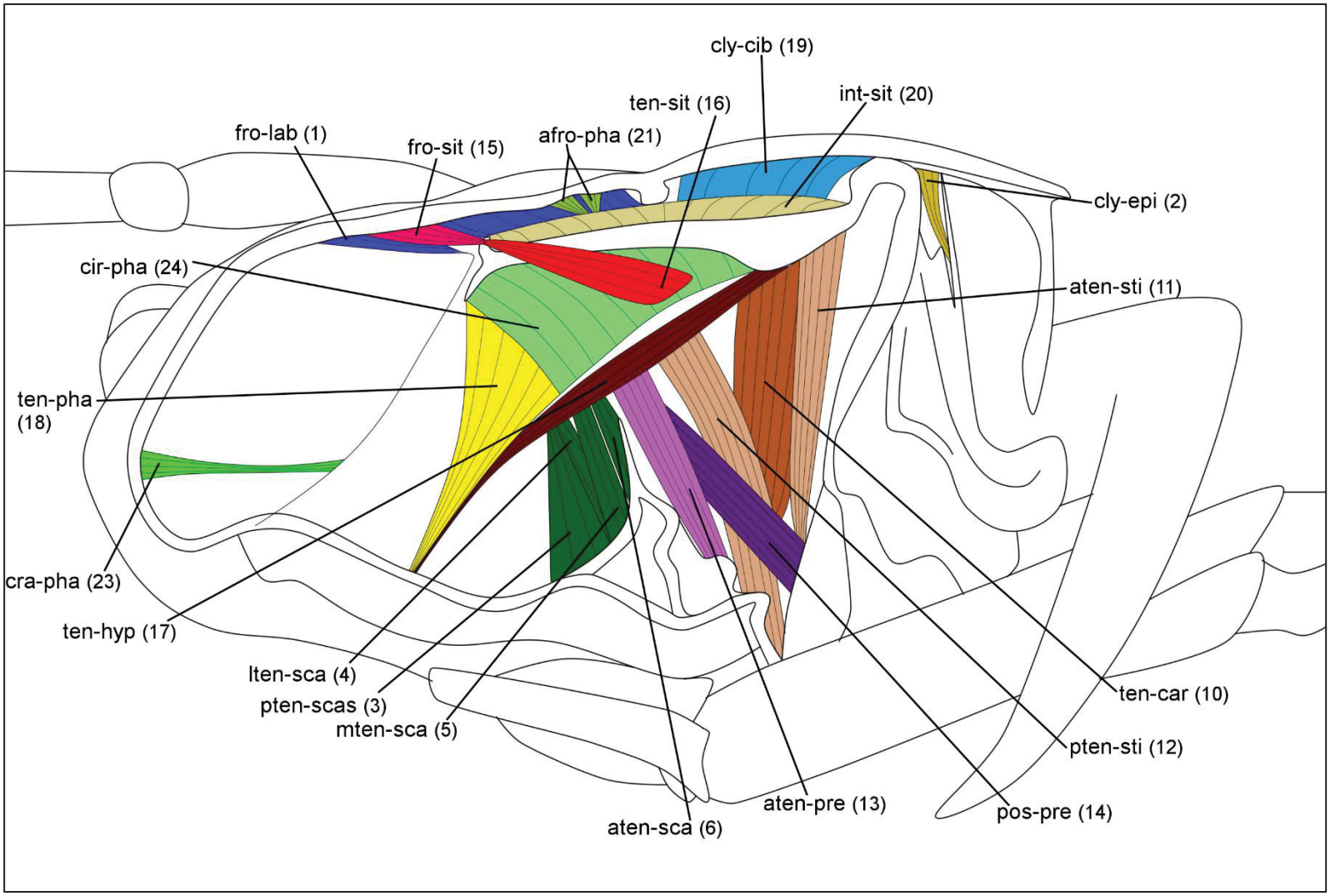

FIGURE 57. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELISSOPTILA AUREOCINCTA* URBAN, 1968 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

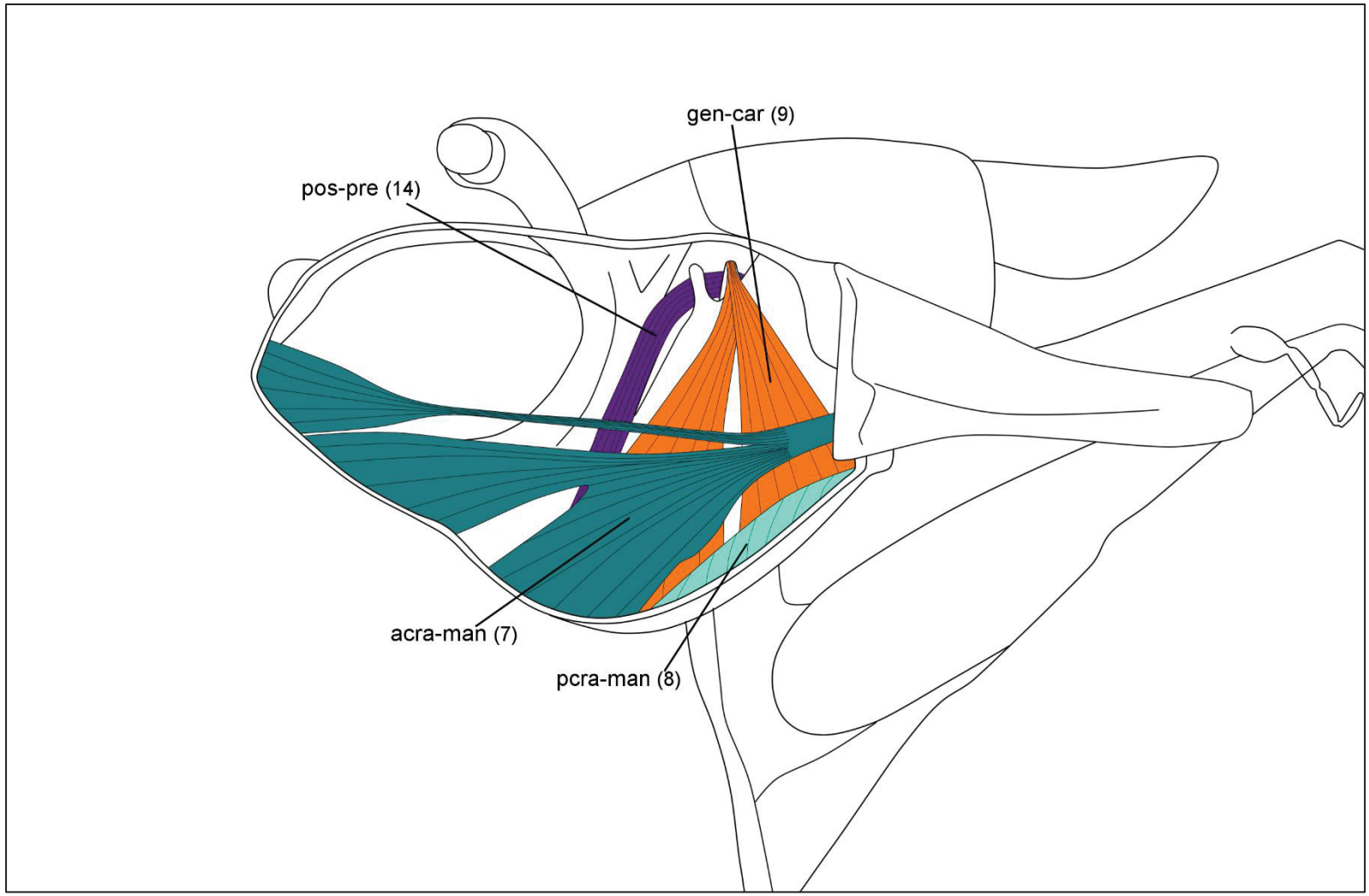

FIGURE 58. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *THYGATER (THYGATER) ANALIS* (LEPELETIER, 1841) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

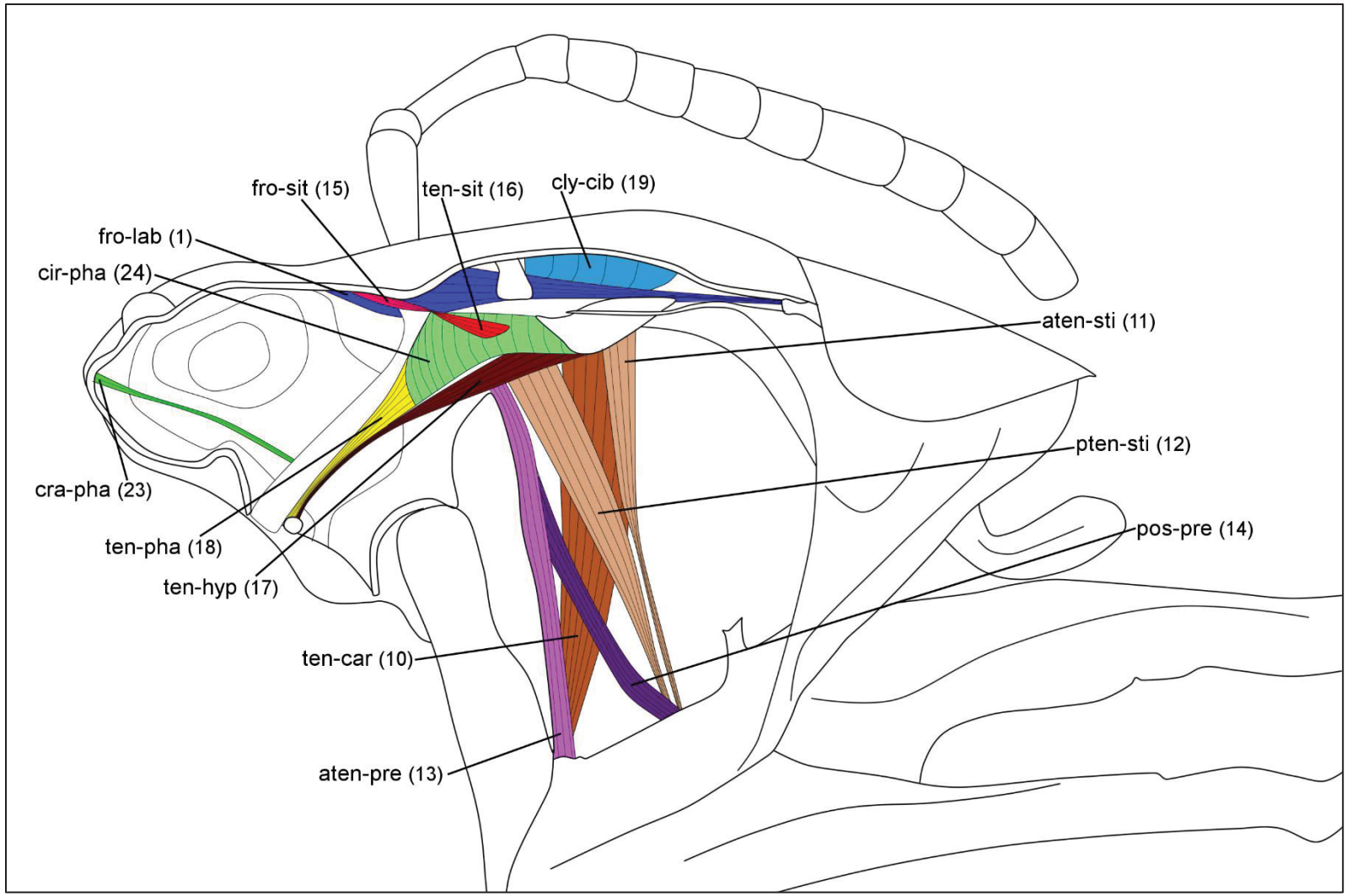

FIGURE 59. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *THYGATER (THYGATER) ANALIS* (LEPELETIER, 1841) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

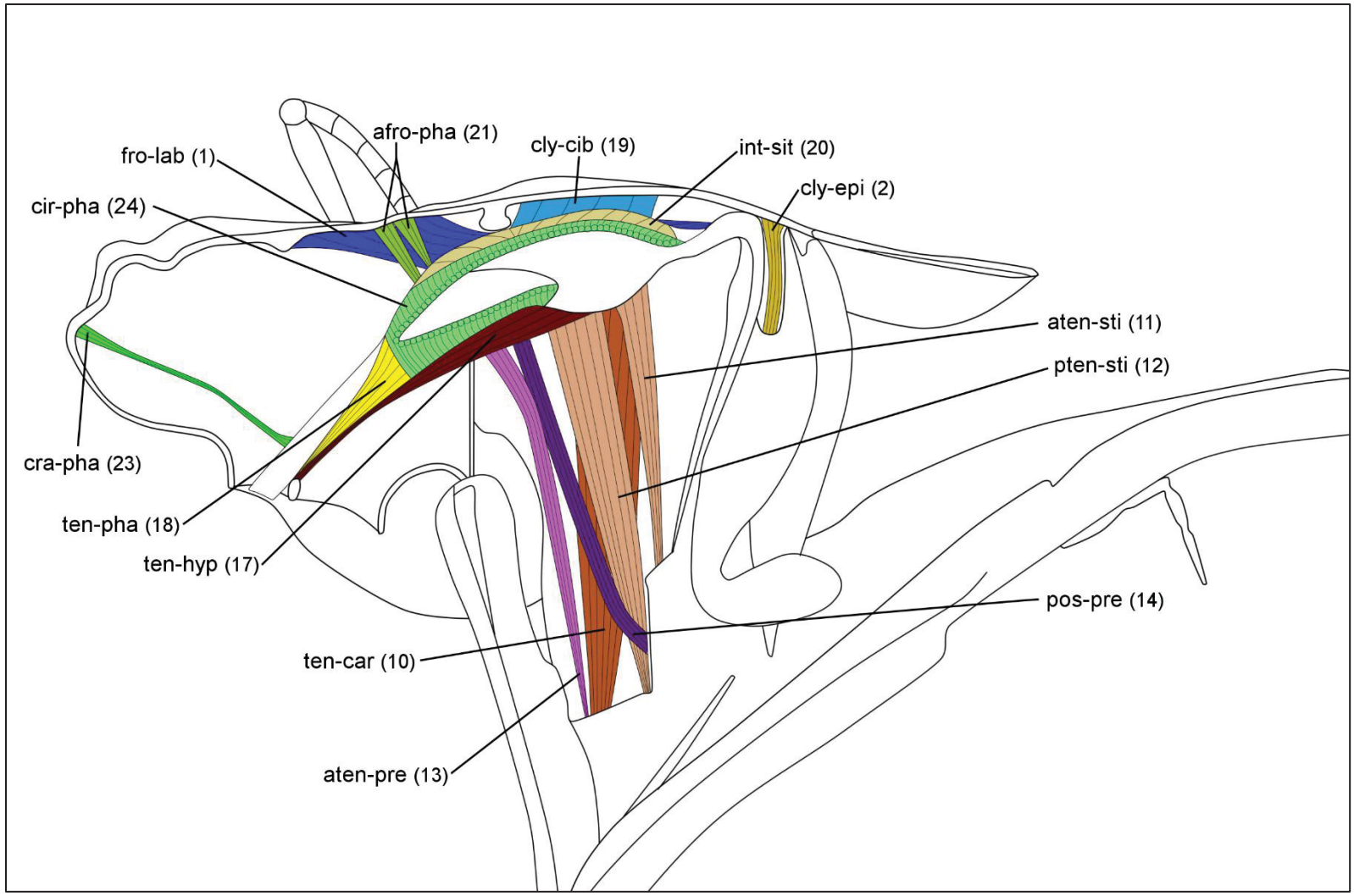

FIGURE 60. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *THYGATER (THYGATER) ANALIS* (LEPELETIER, 1841) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

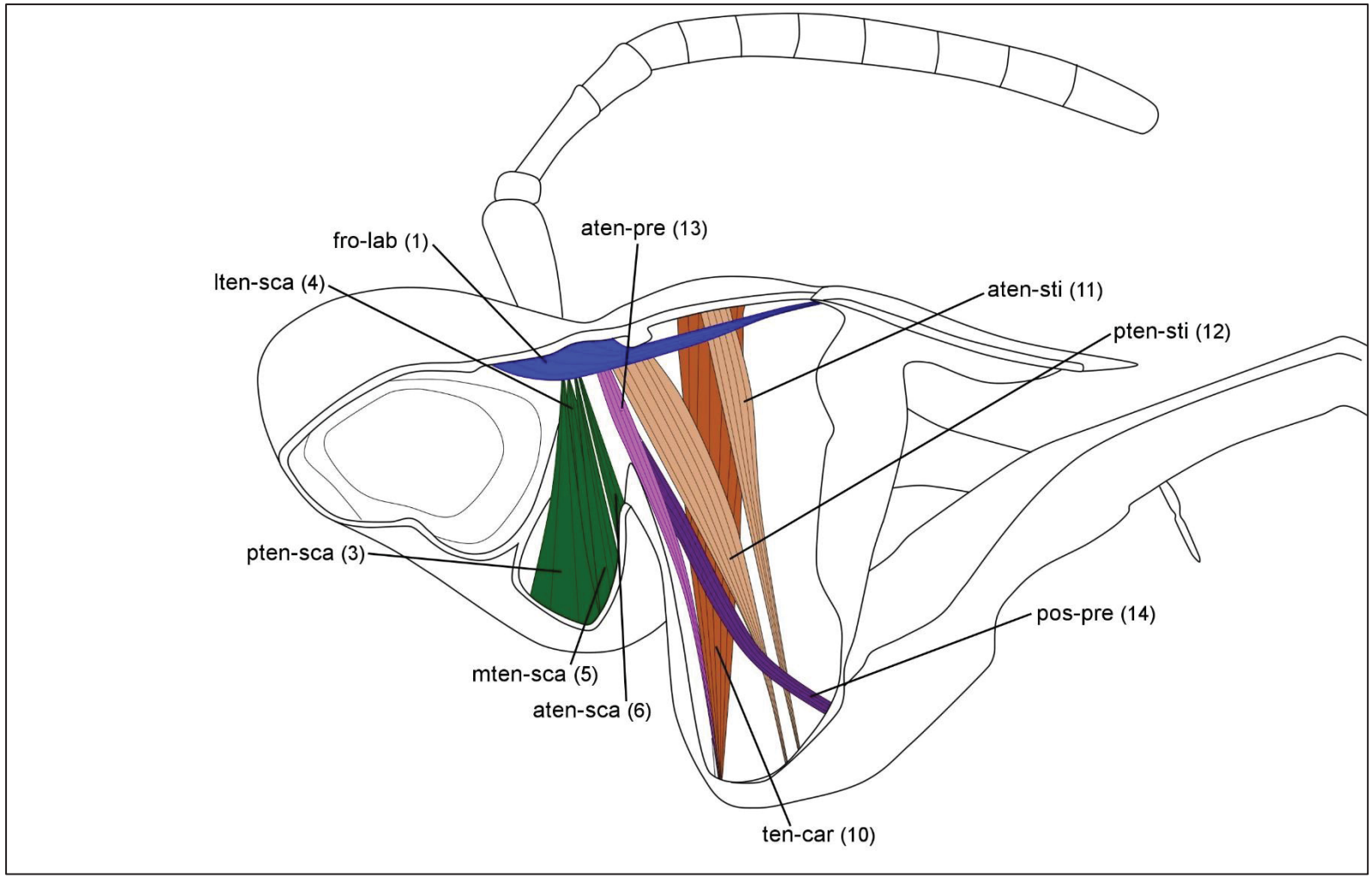

FIGURE 61. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *THYGATER (THYGATER) ANALIS* (LEPELETIER, 1841) (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

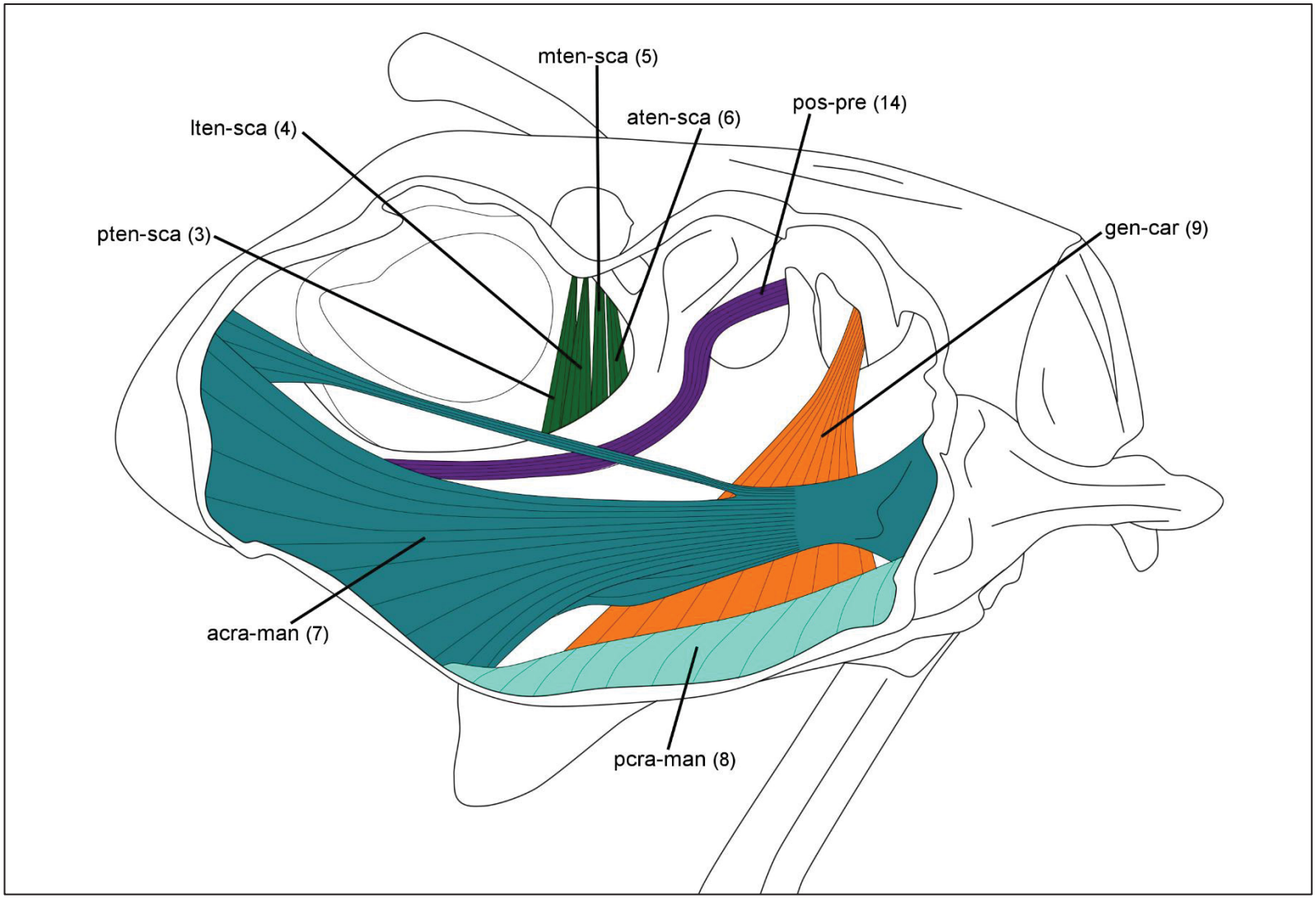

FIGURE 62. EXTRINSIC MUSCLES OF THE HEAD OF MALE *EULAEMA (APEULAEMA) CINGULATA* (FABRICIUS, 1804) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

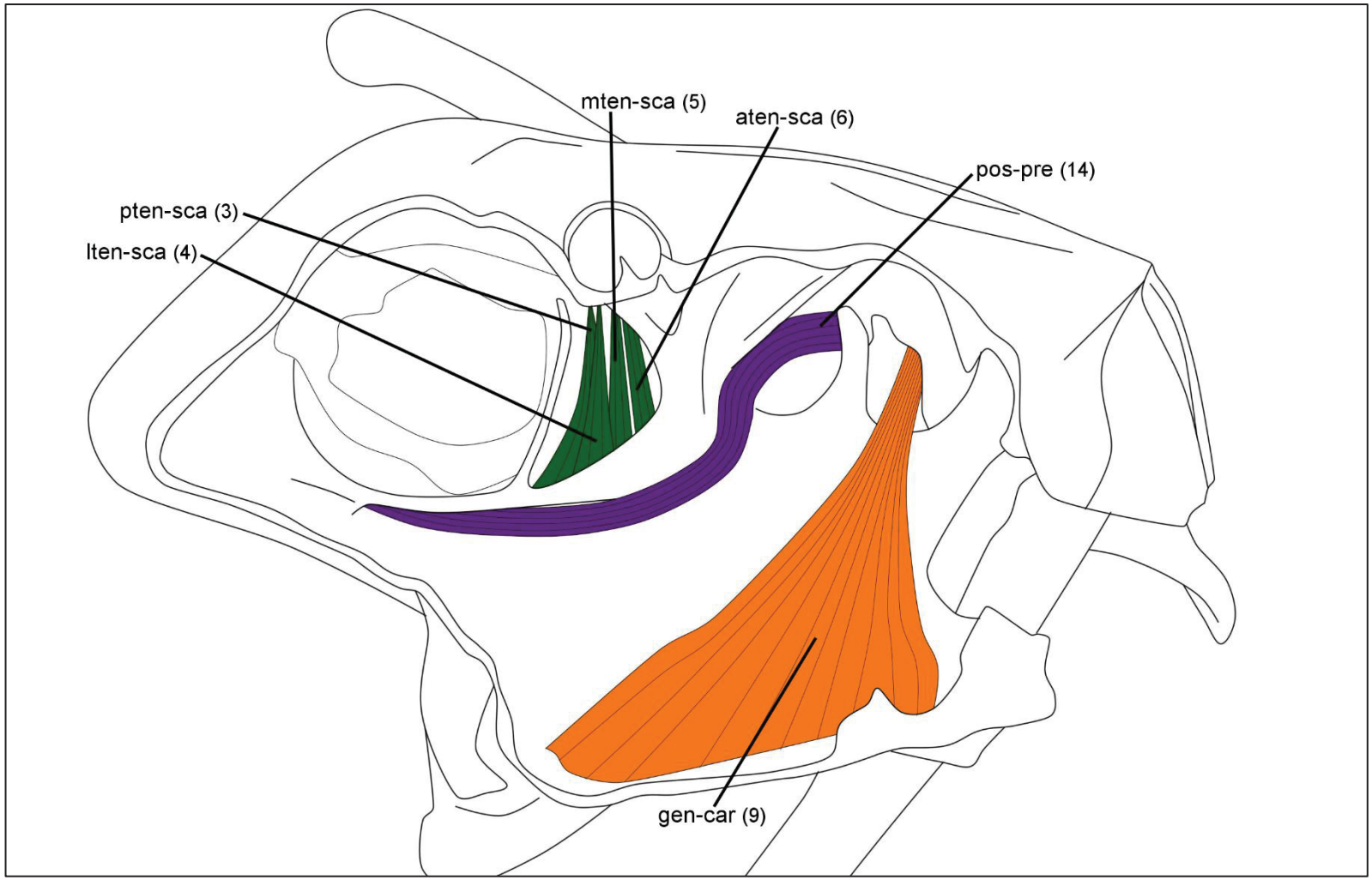

FIGURE 63. EXTRINSIC MUSCLES OF THE HEAD OF MALE *EULAEMA (APEULAEMA) CINGULATA* (FABRICIUS, 1804) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

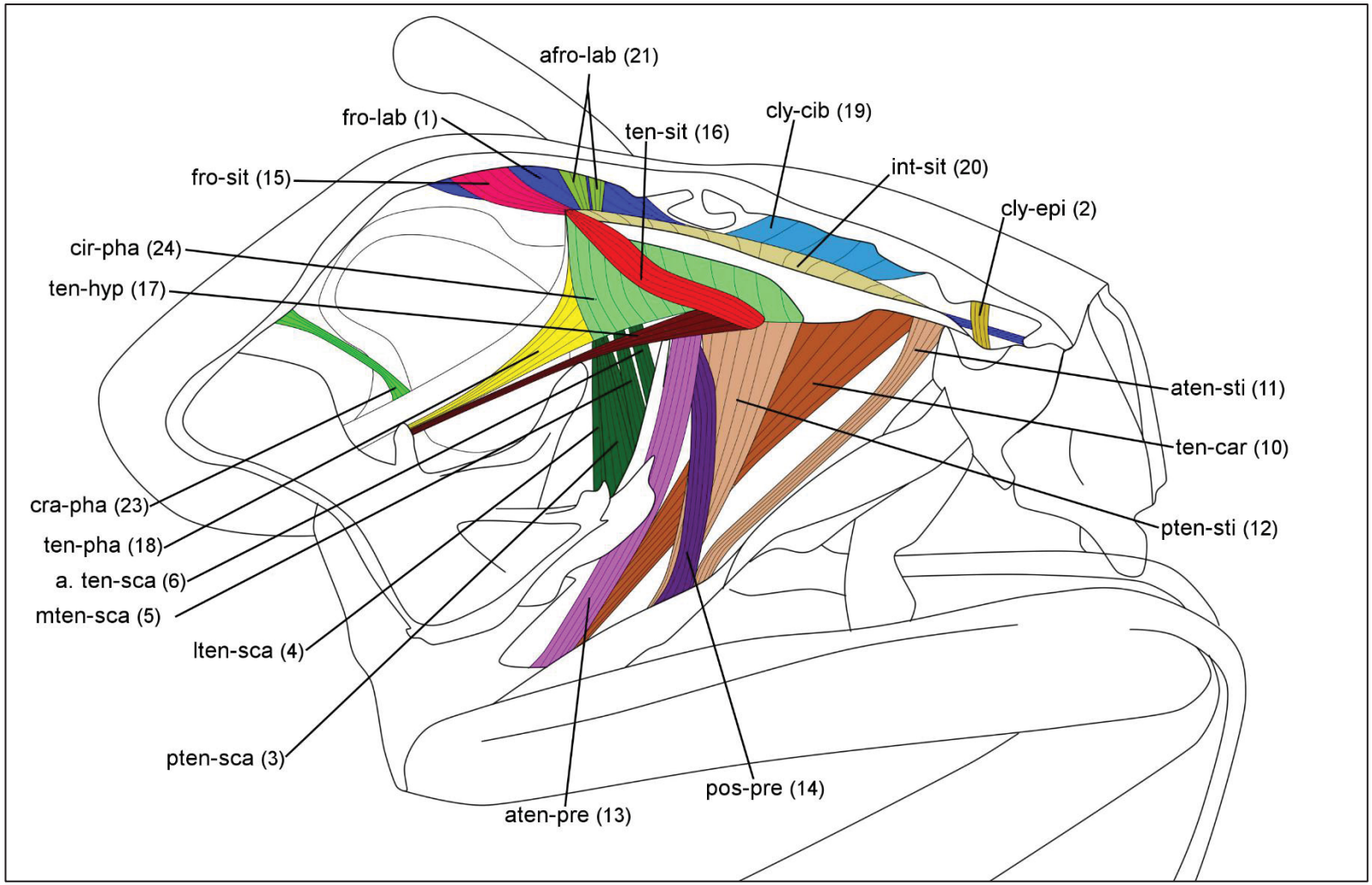

FIGURE 64. EXTRINSIC MUSCLES OF THE HEAD OF MALE *EULAEMA (APEULAEMA) CINGULATA* (FABRICIUS, 1804) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

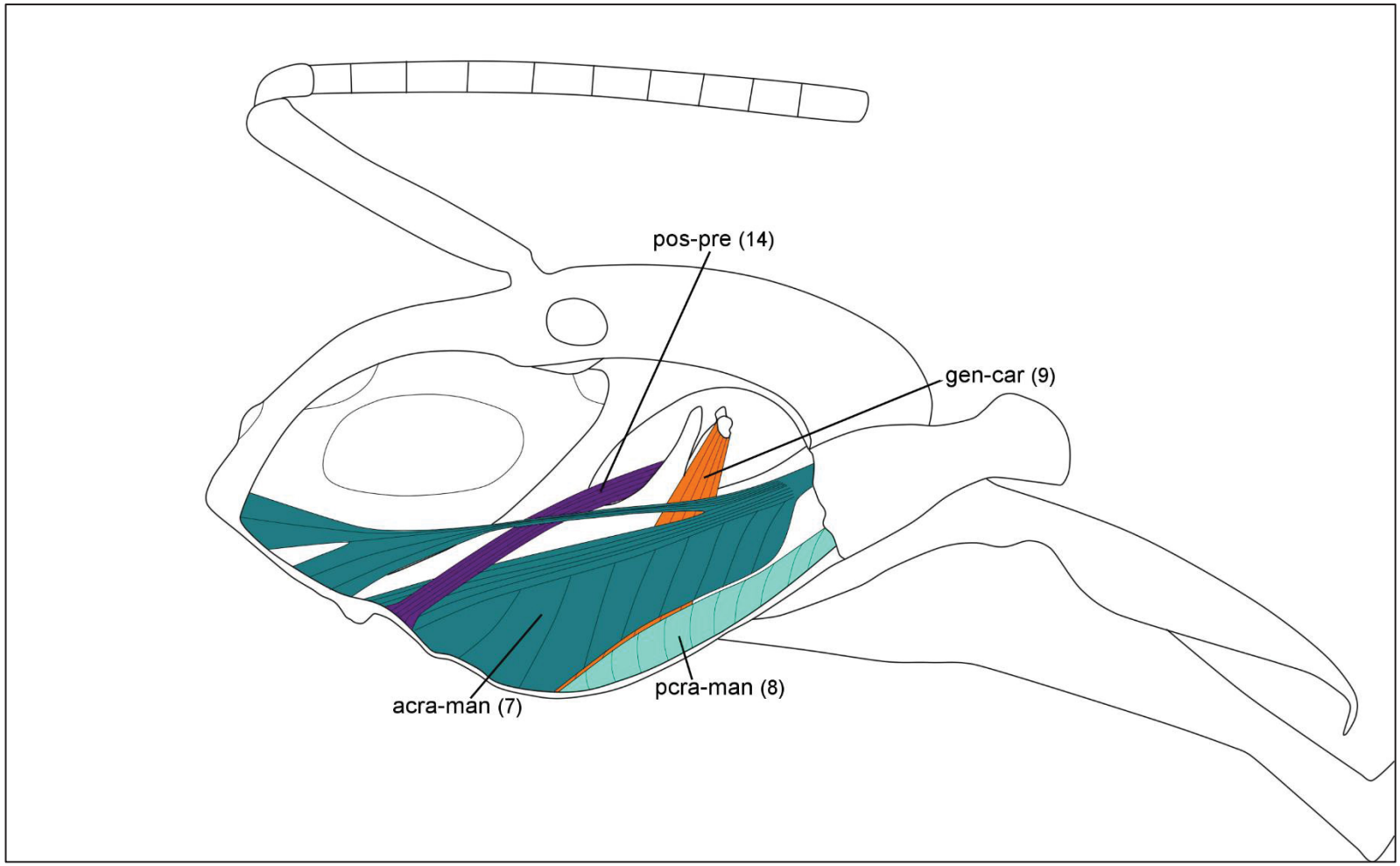

FIGURE 65. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELIPONA (MELIPONA) QUADRIFASCIATA* LEPELETIER, 1836 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

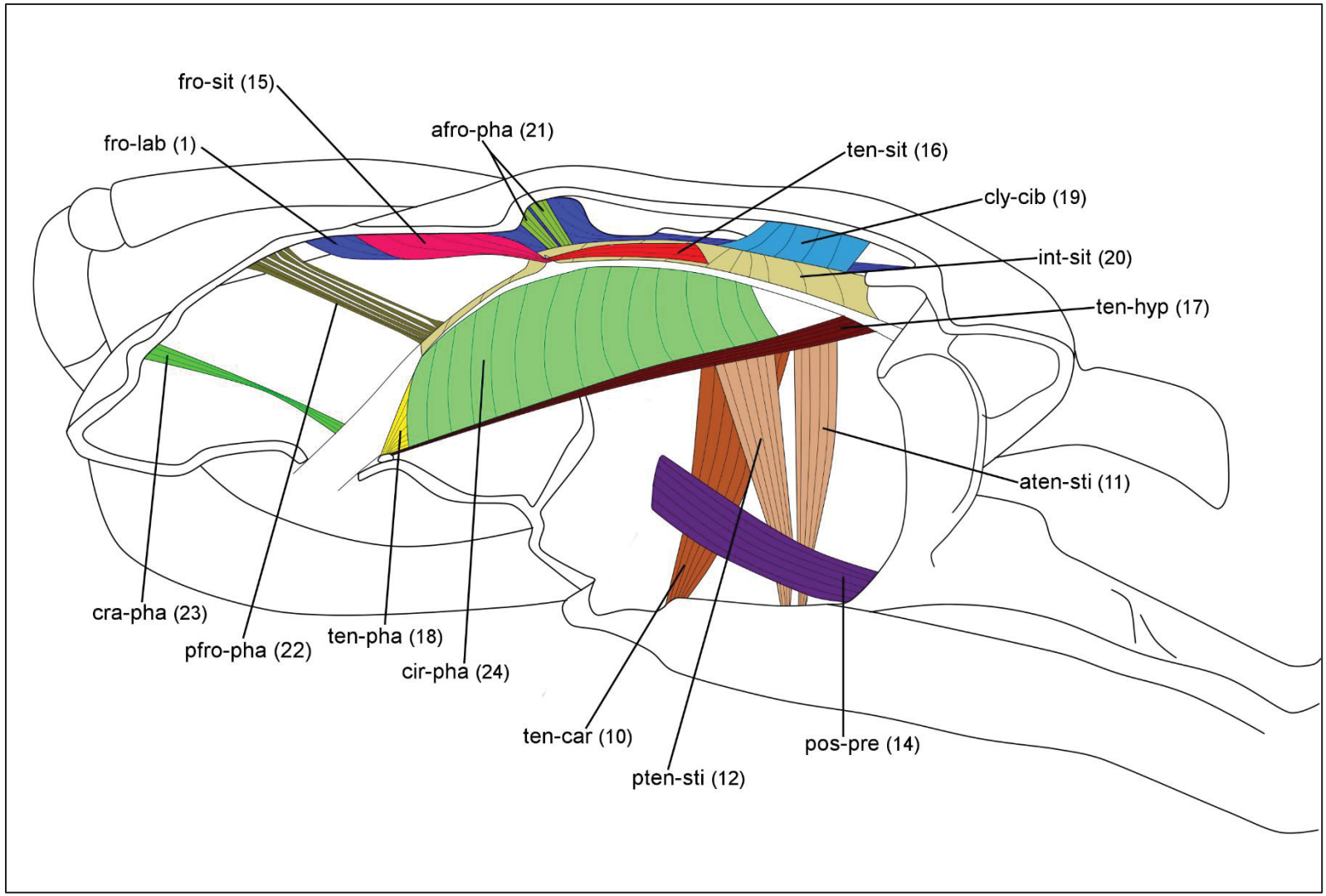

FIGURE 66. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELIPONA (MELIPONA) QUADRIFASCIATA* LEPELETIER, 1836 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

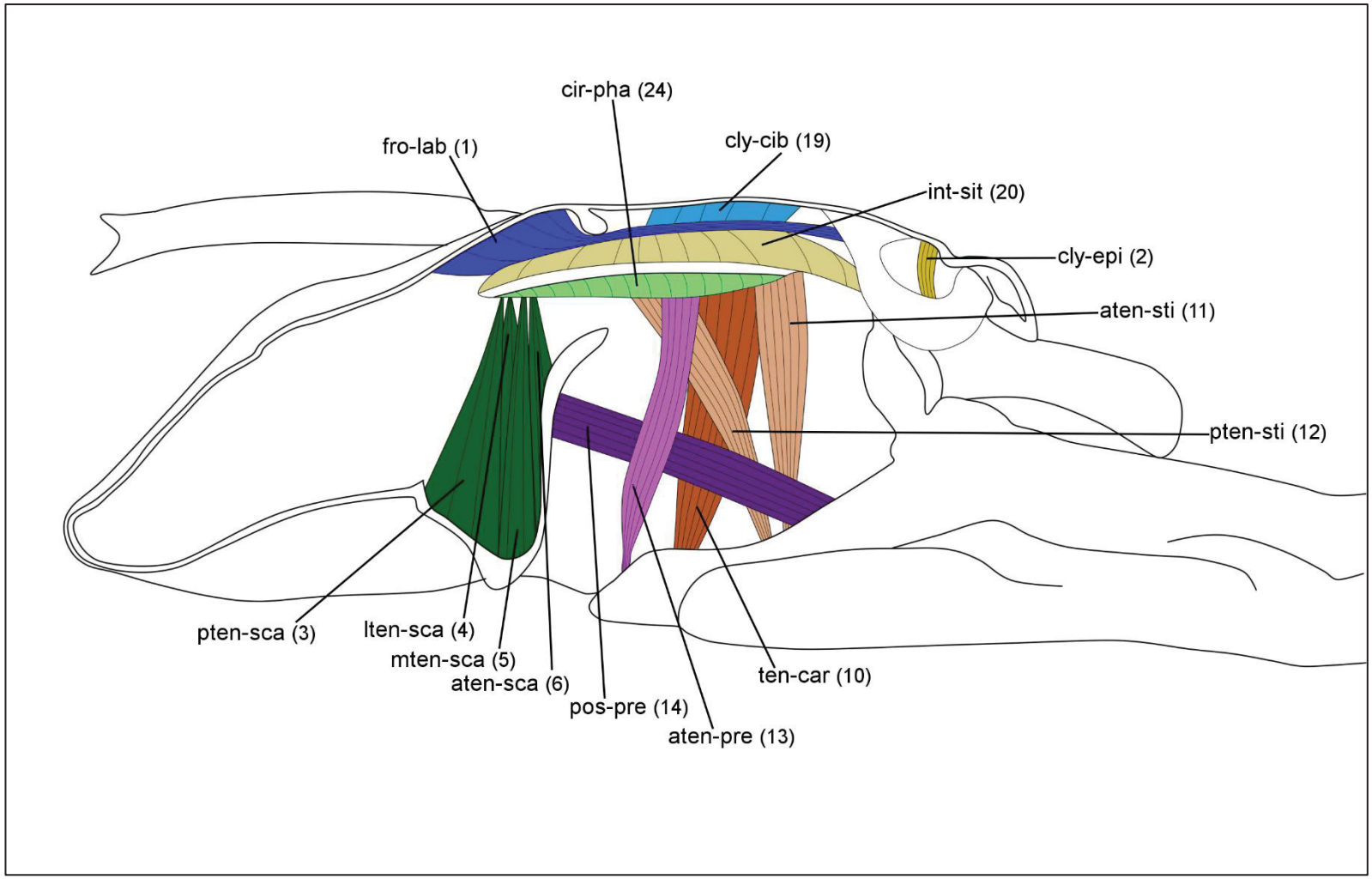

FIGURE 67. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELIPONA (MELIPONA) QUADRIFASCIATA* LEPELETIER, 1836 (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

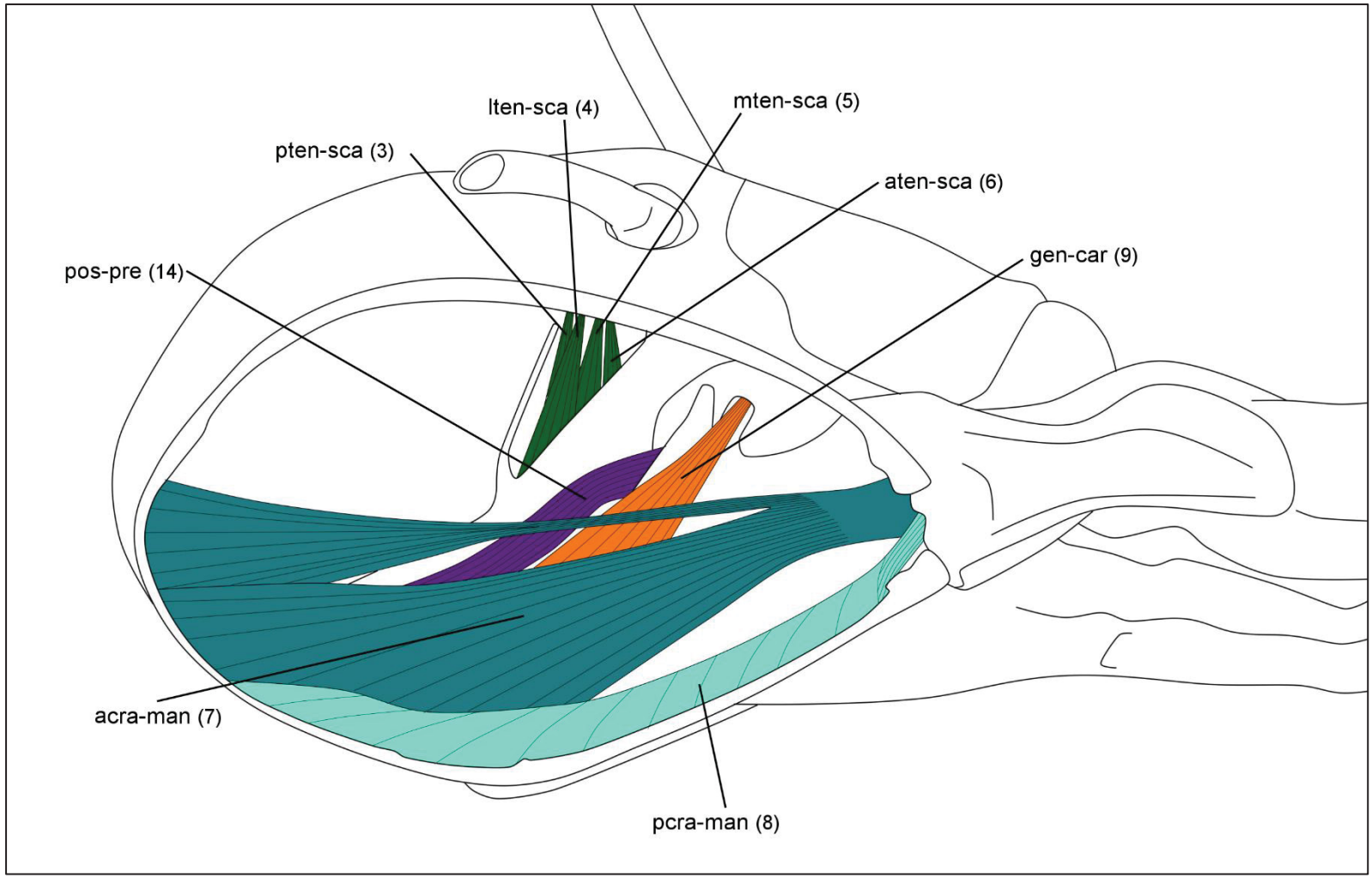

FIGURE 68. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *SCAPTOTRIGONA BIPUNCTATA* (LEPELETIER, 1836) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

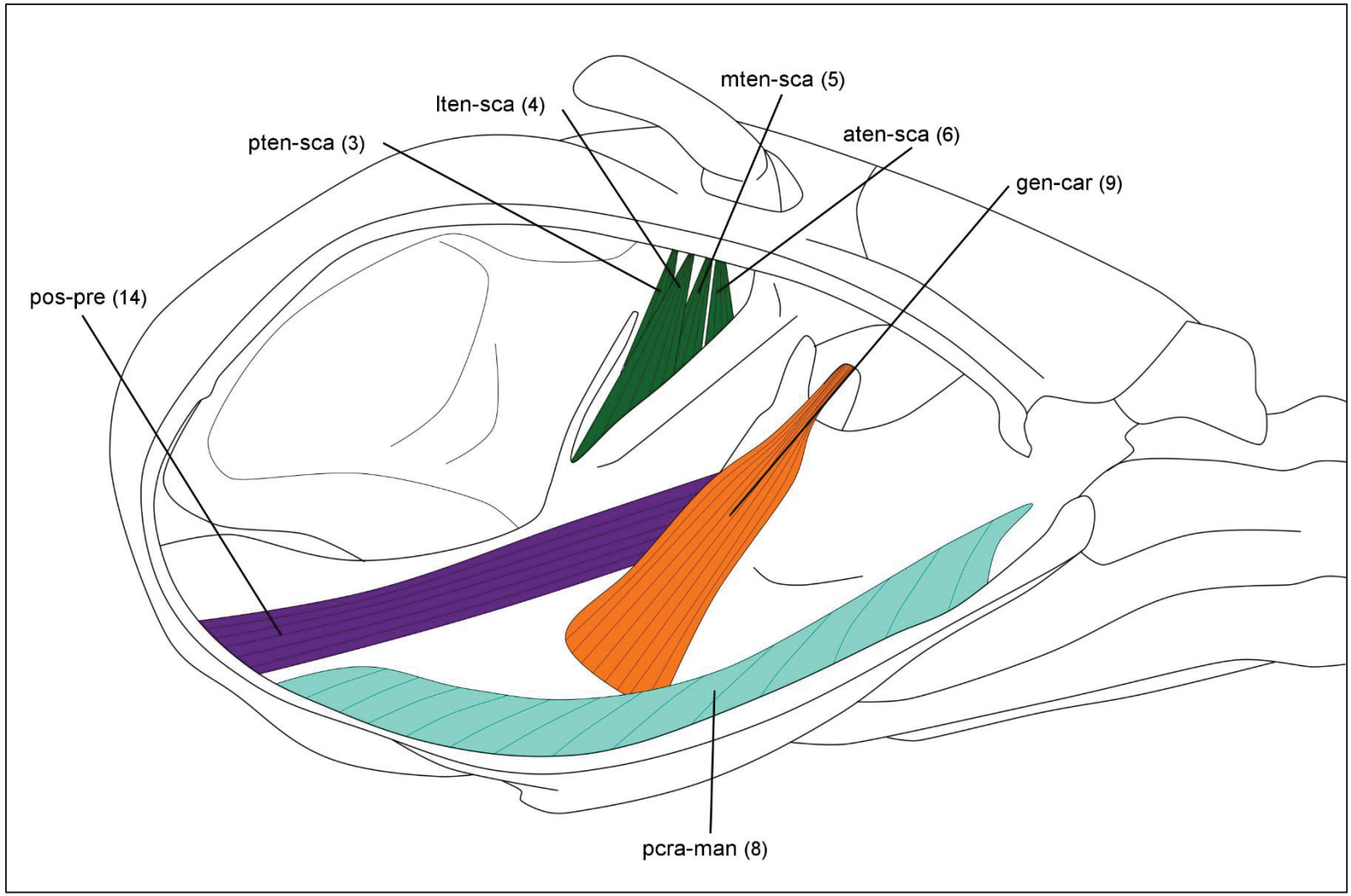

FIGURE 69. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *SCAPTOTRIGONA BIPUNCTATA* (LEPELETIER, 1836) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

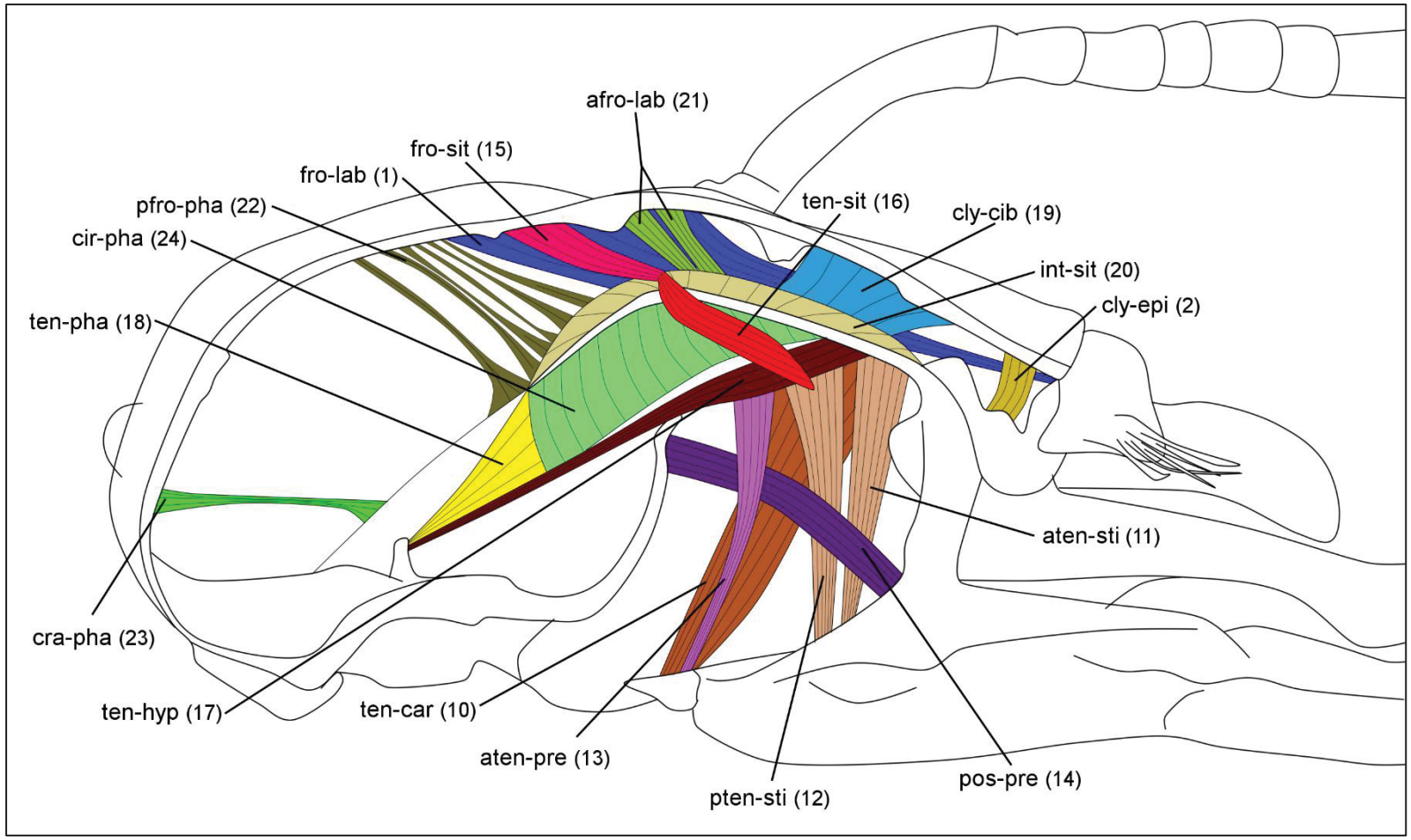

FIGURE 70. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *SCAPTOTRIGONA BIPUNCTATA* (LEPELETIER, 1836) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

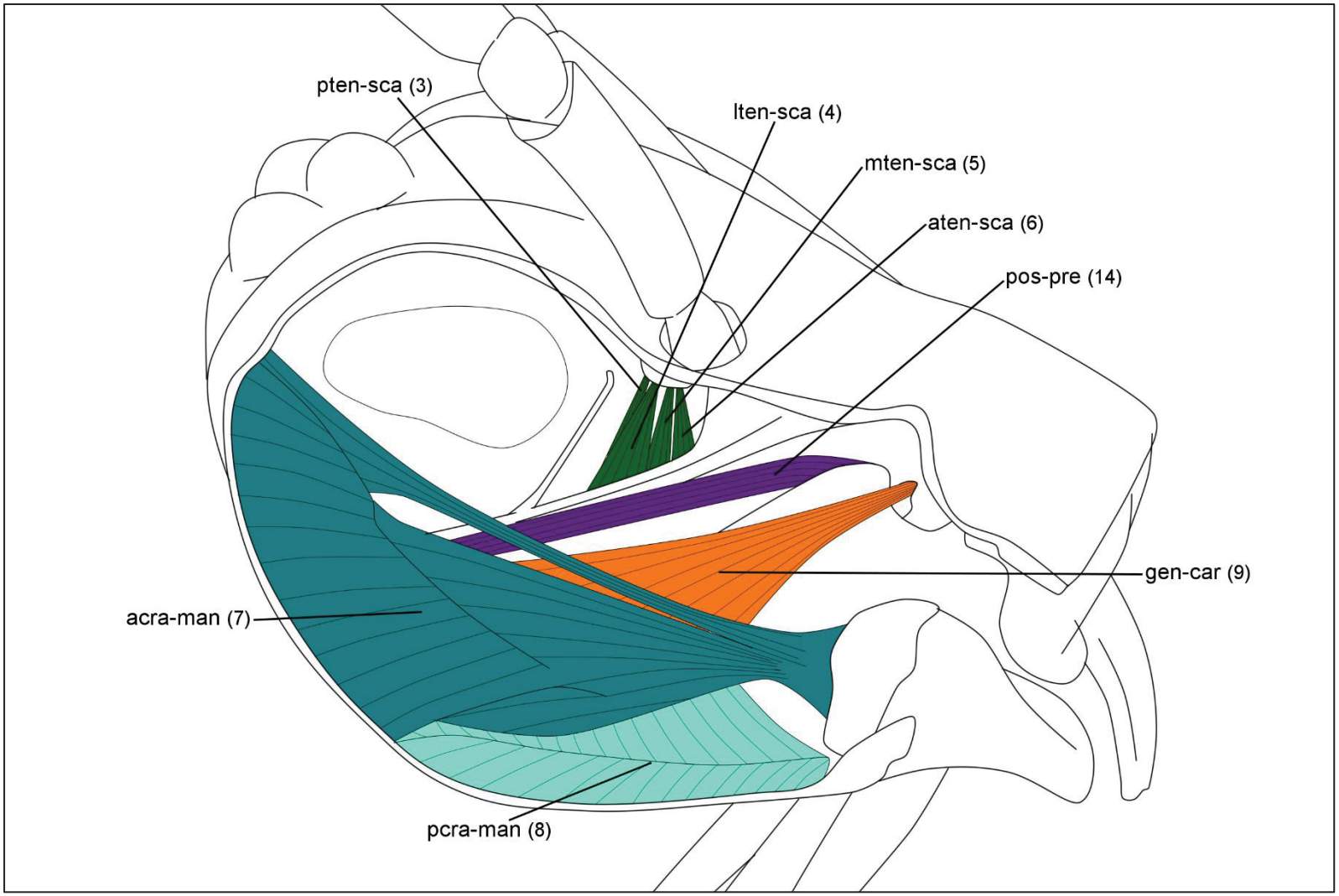

FIGURE 71. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PROTOSIRIS MCGINLEYI* (SHANKS, 1986) (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

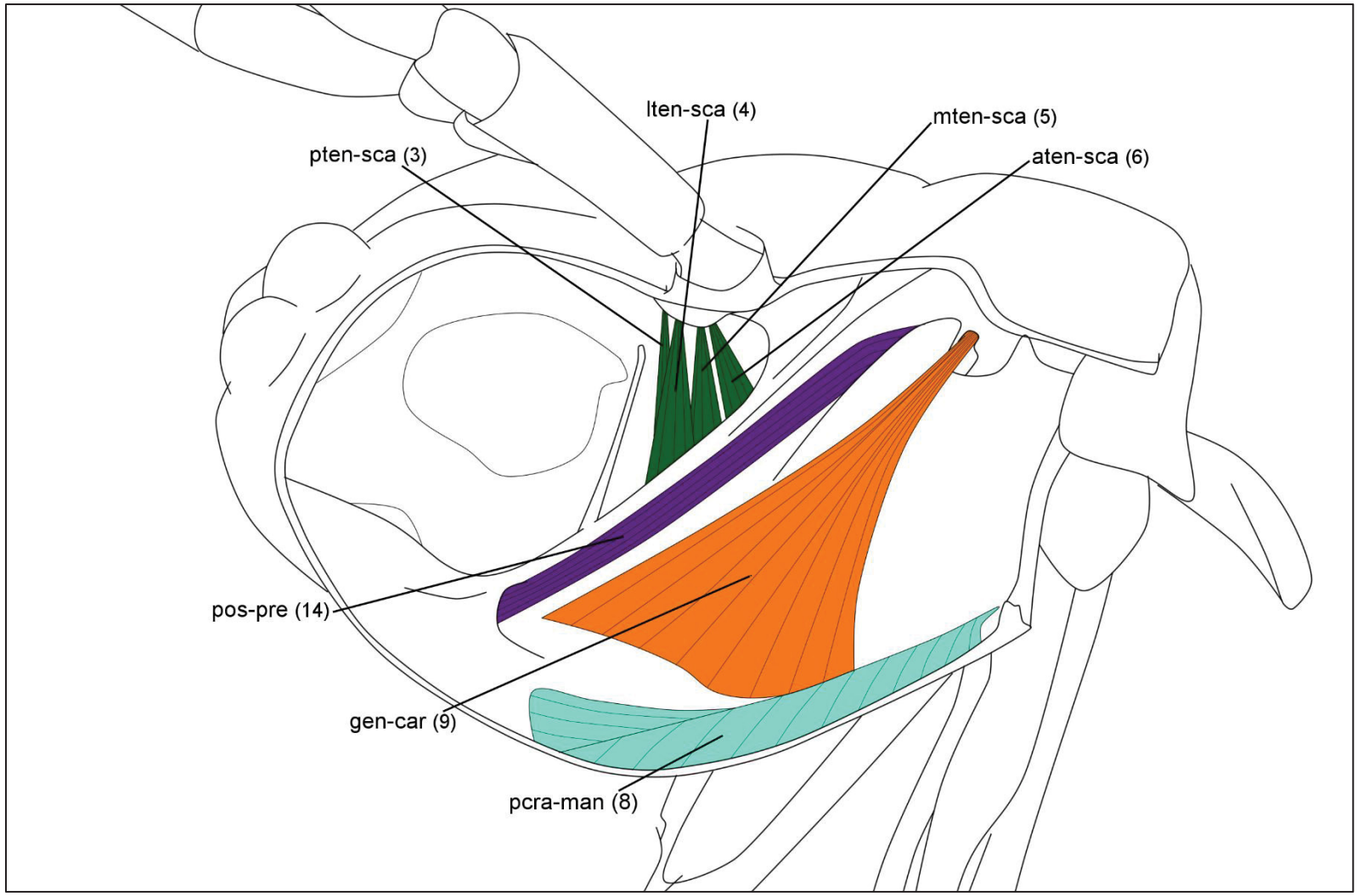

FIGURE 72. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PROTOSIRIS MCGINLEYI* (SHANKS, 1986) (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

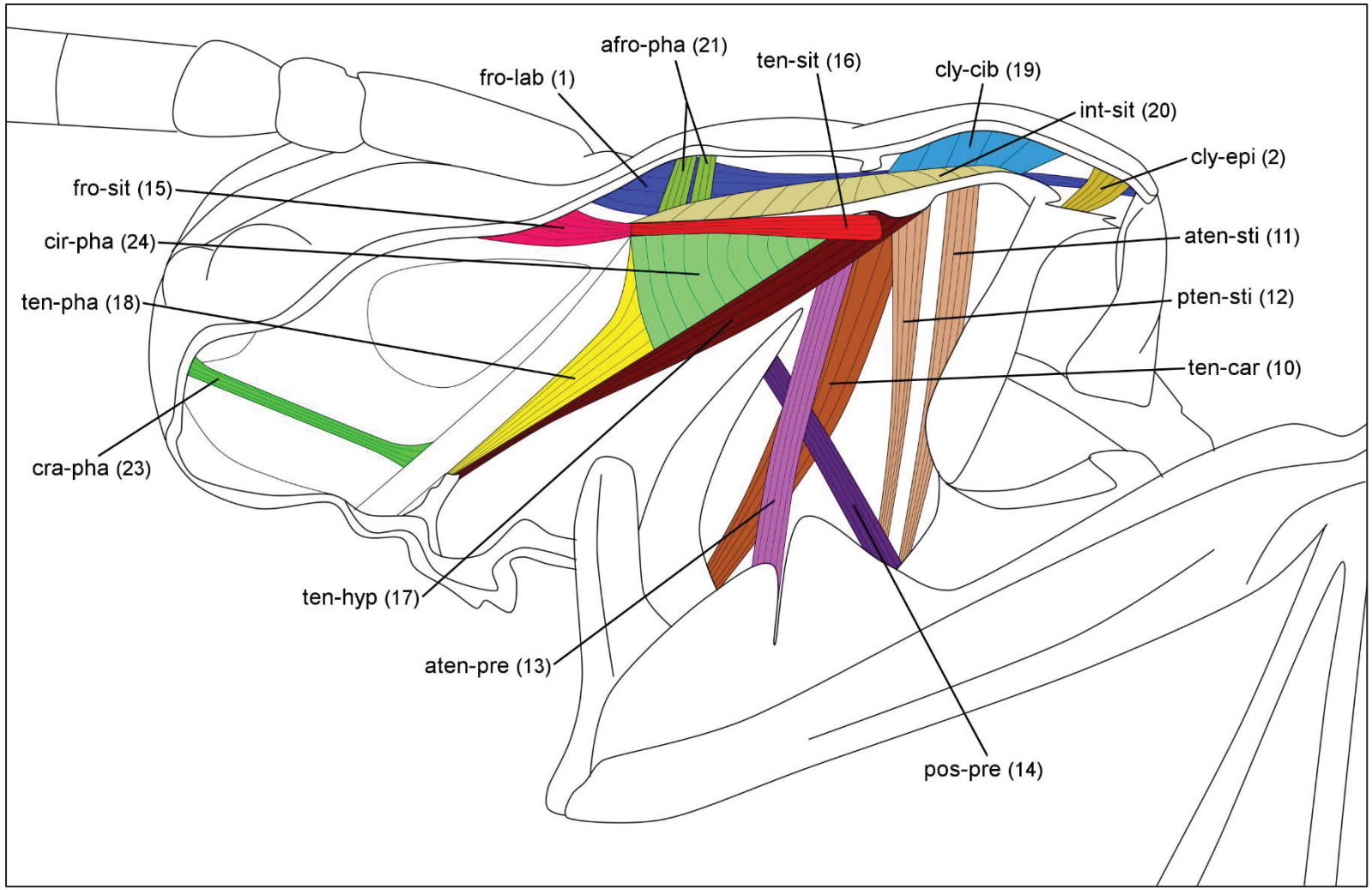

FIGURE 73. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PROTOSIRIS MCGINLEYI* (SHANKS, 1986) (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

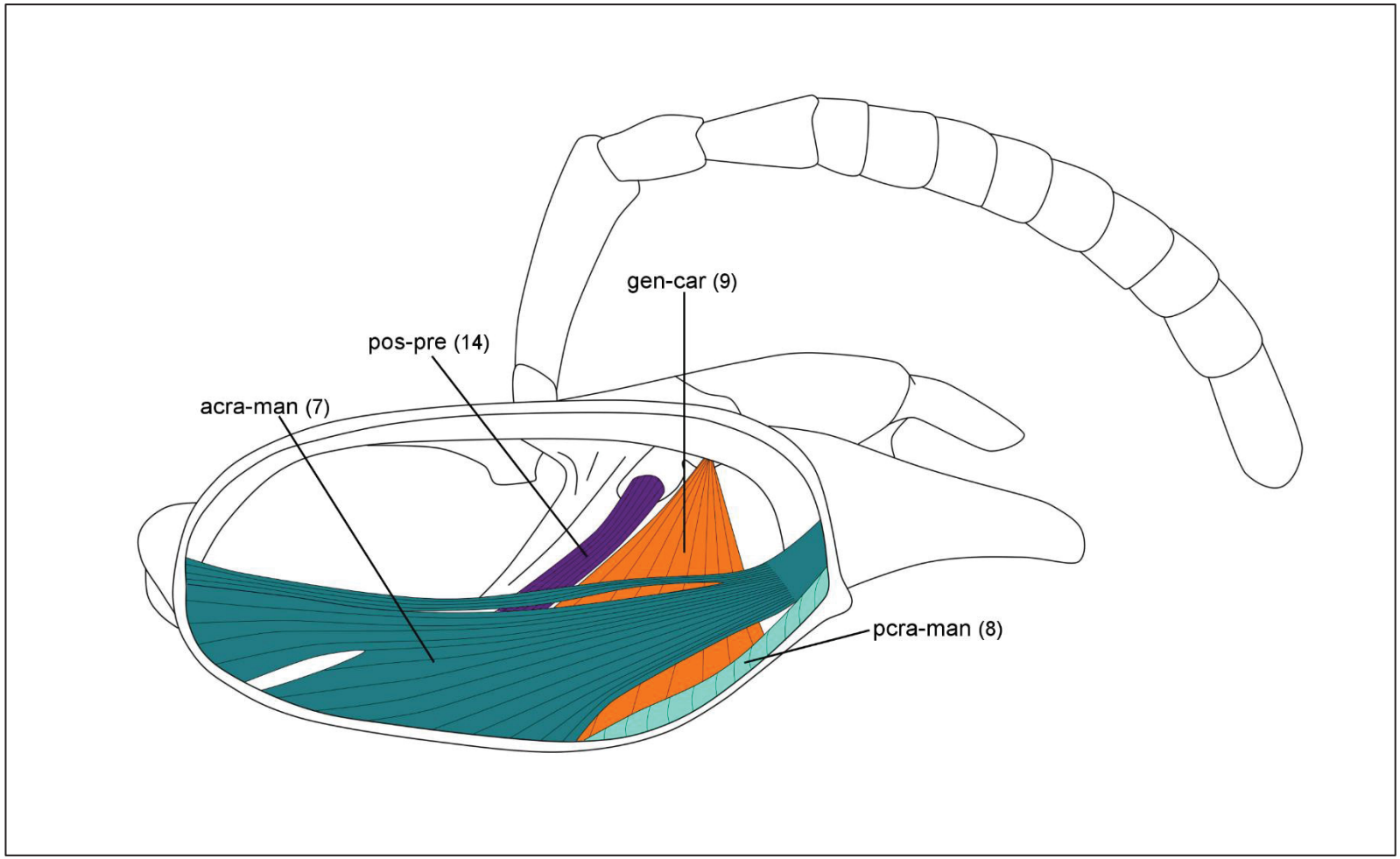

FIGURE 74. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ARHYSOCEBLE* SP. (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

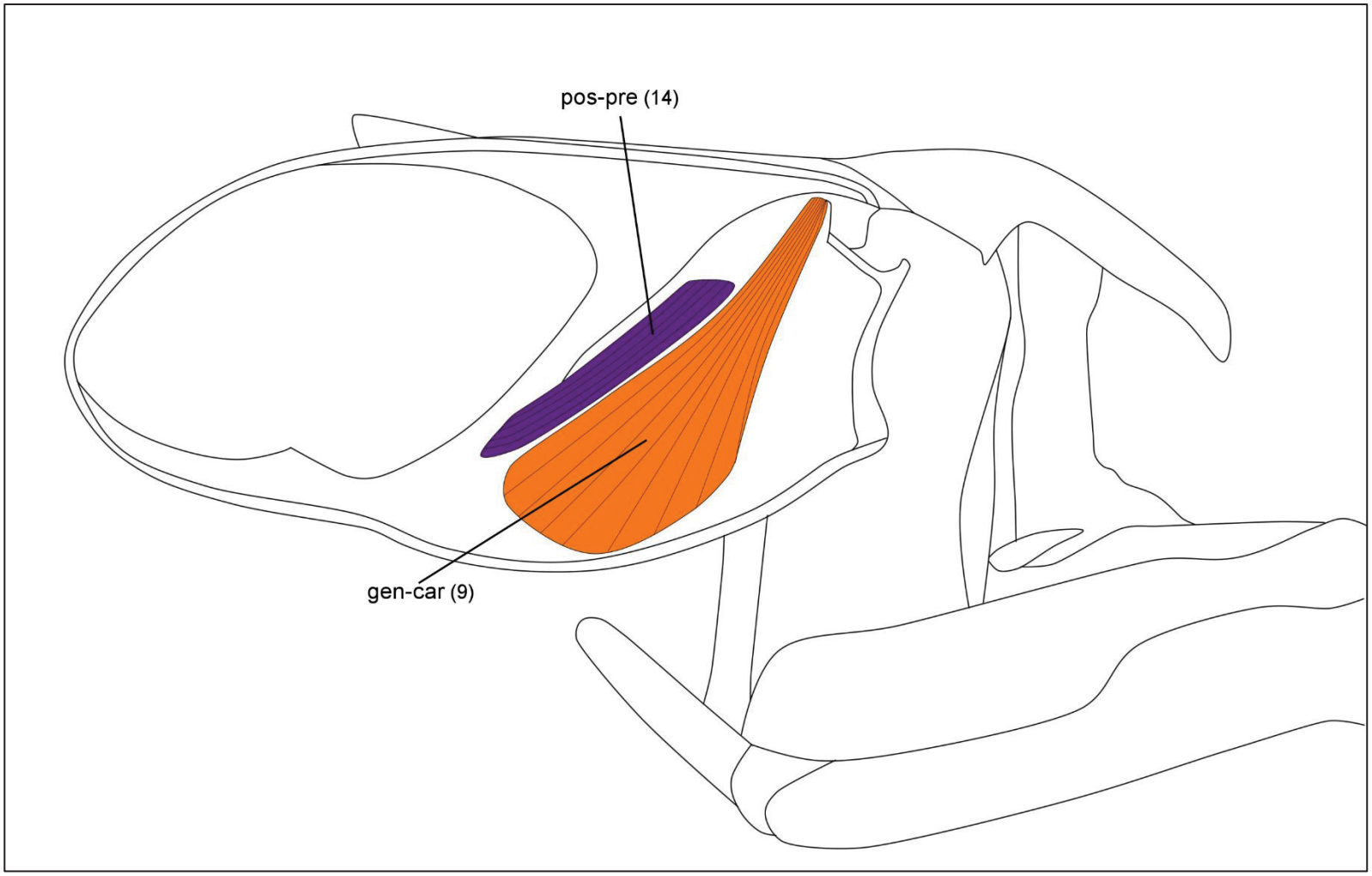

FIGURE 75. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ARHYSOCEBLE* SP. (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

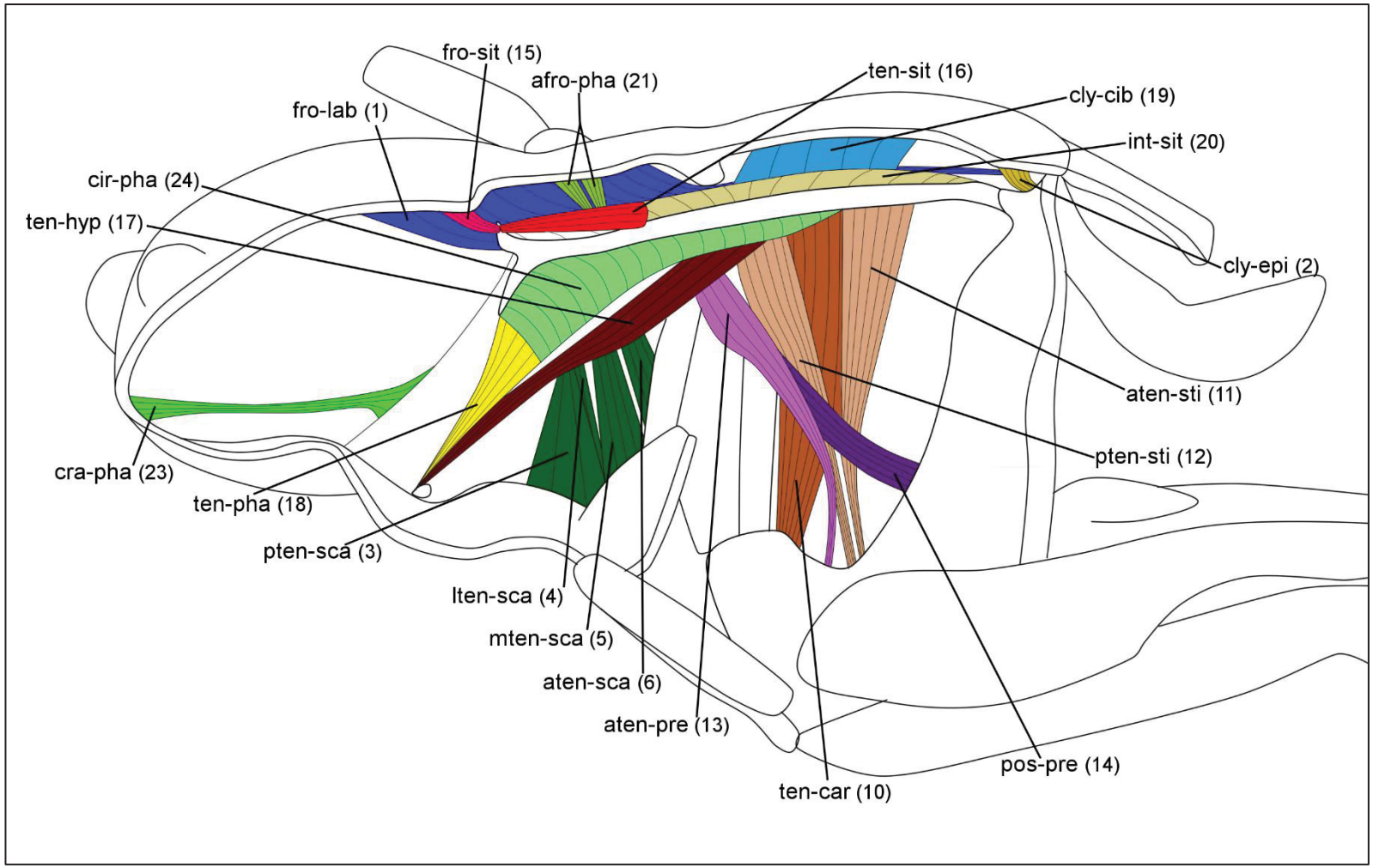

FIGURE 76. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ARHYSOCEBLE* SP. (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

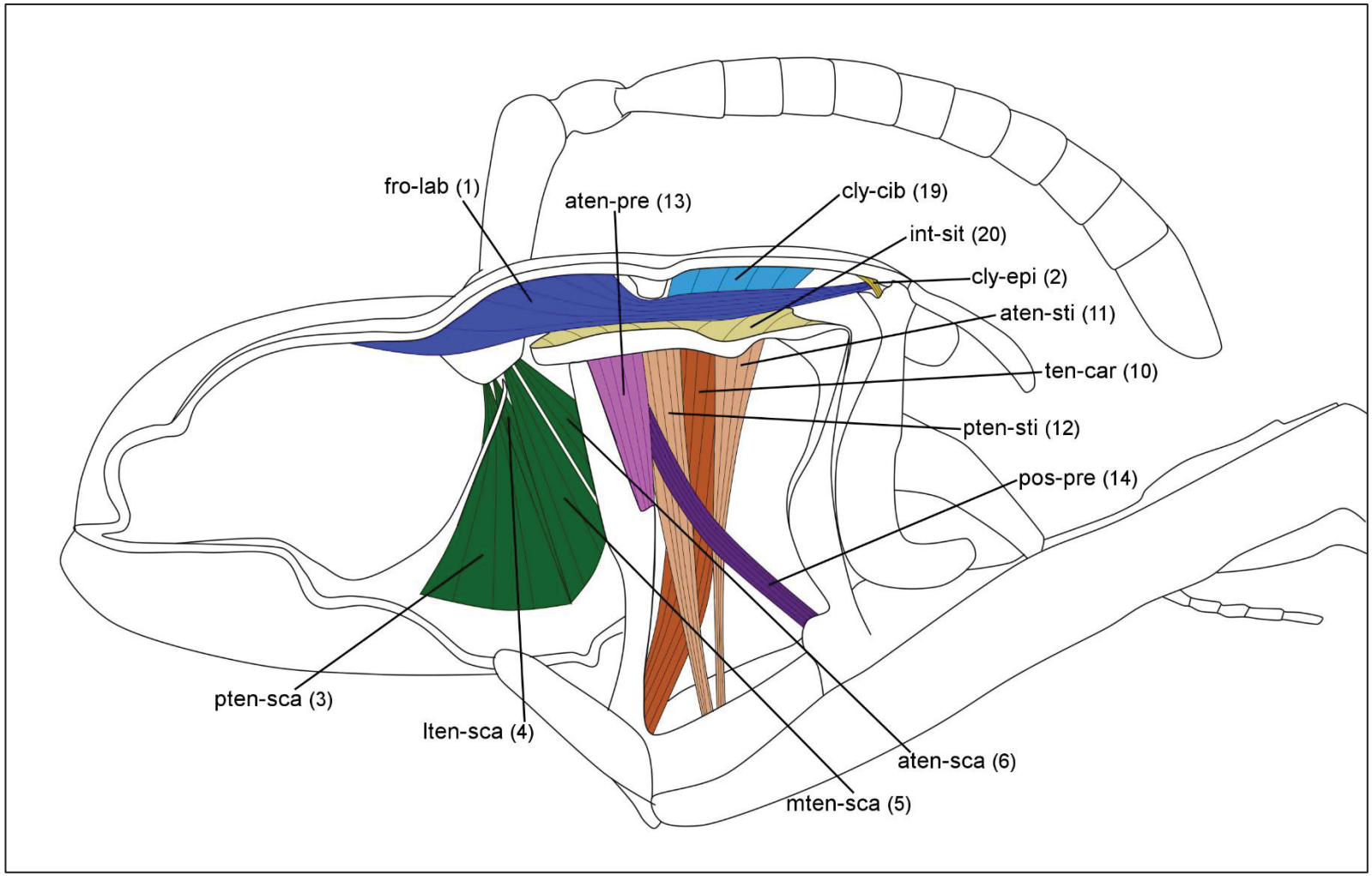

FIGURE 77. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *ARHYSOCEBLE* SP. (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

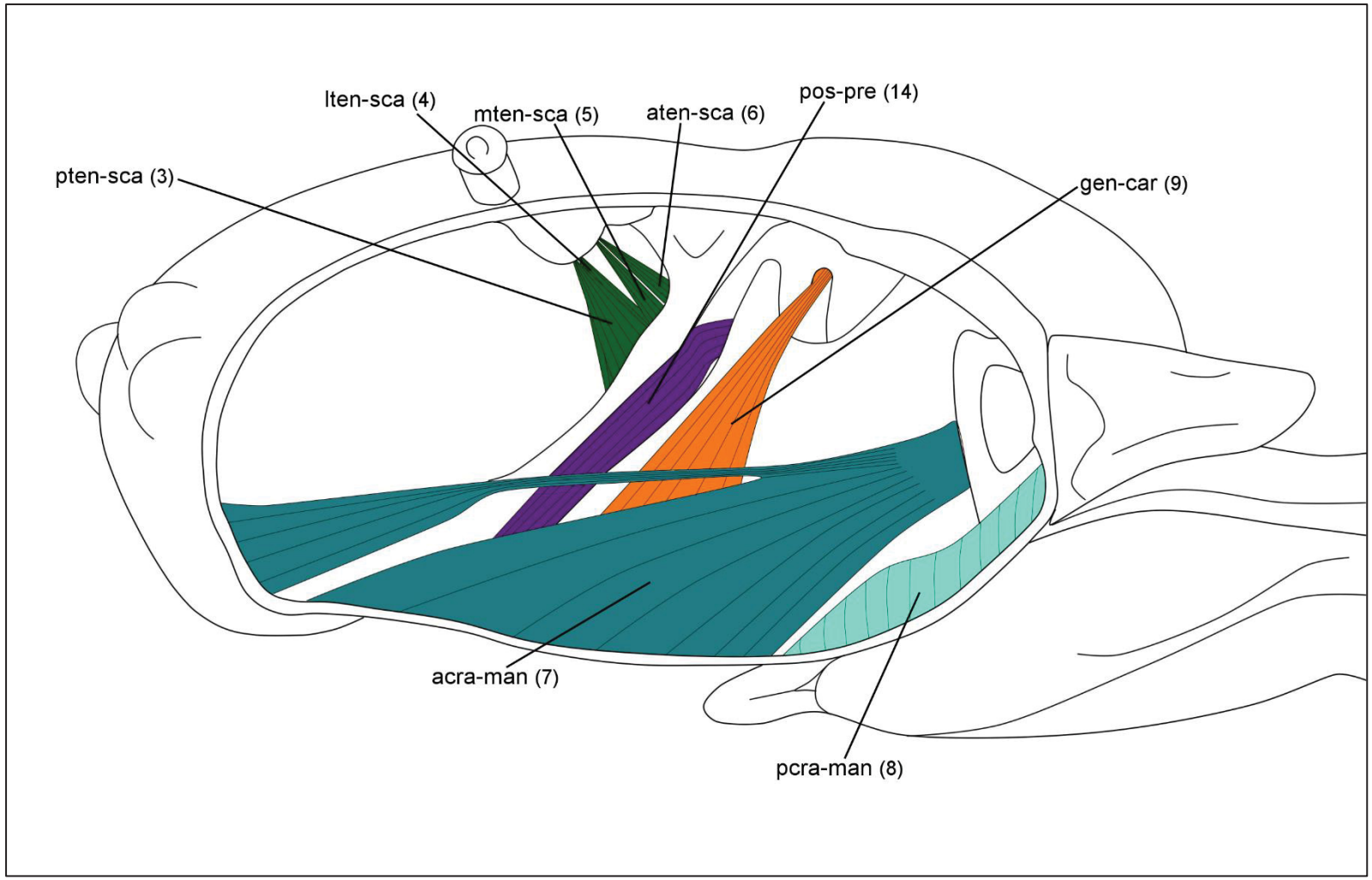

FIGURE 78. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAPEDIA DIVERSIPES* KLUG, 1810 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

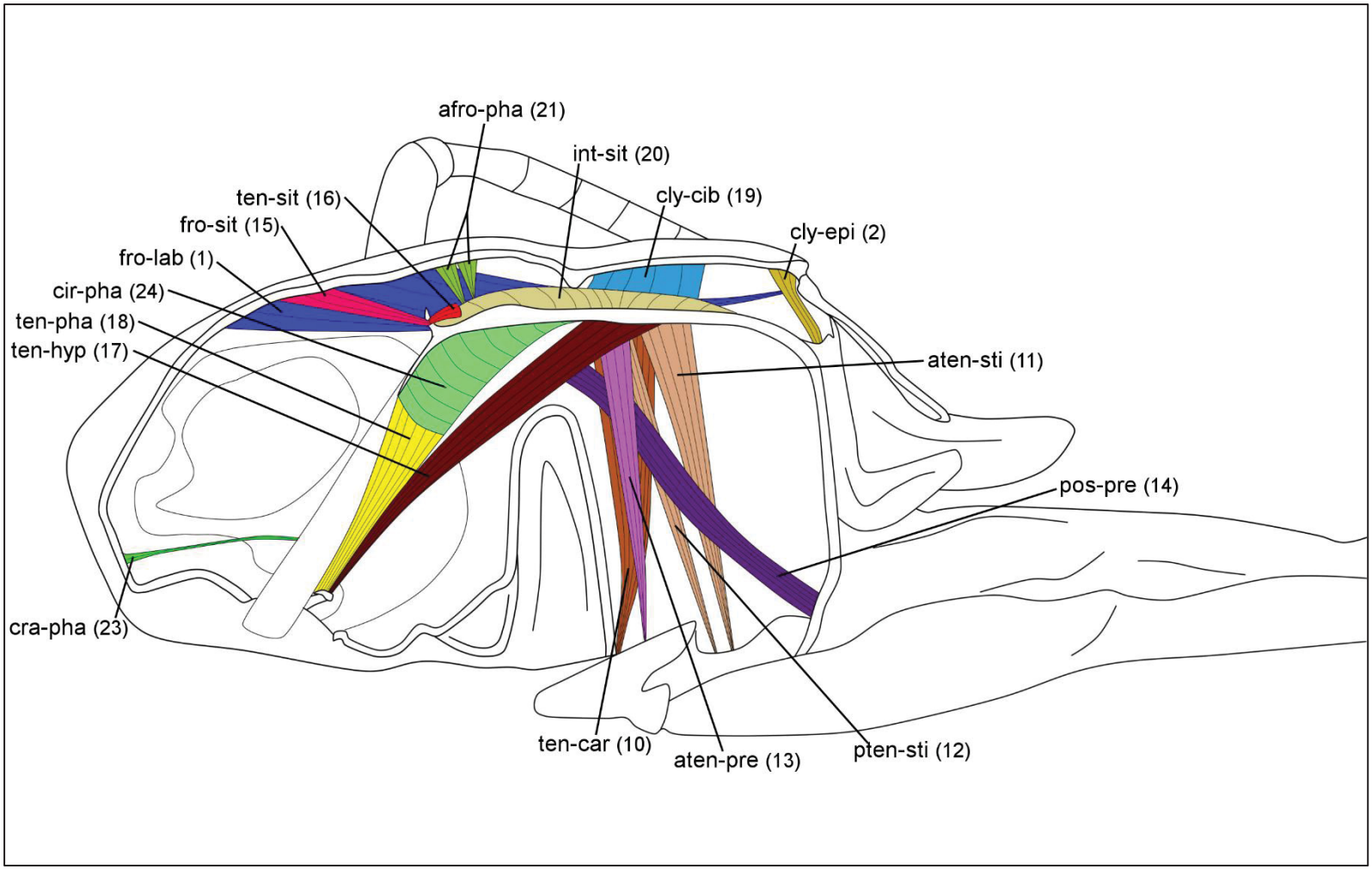

FIGURE 79. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAPEDIA DIVERSIPES* KLUG, 1810 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

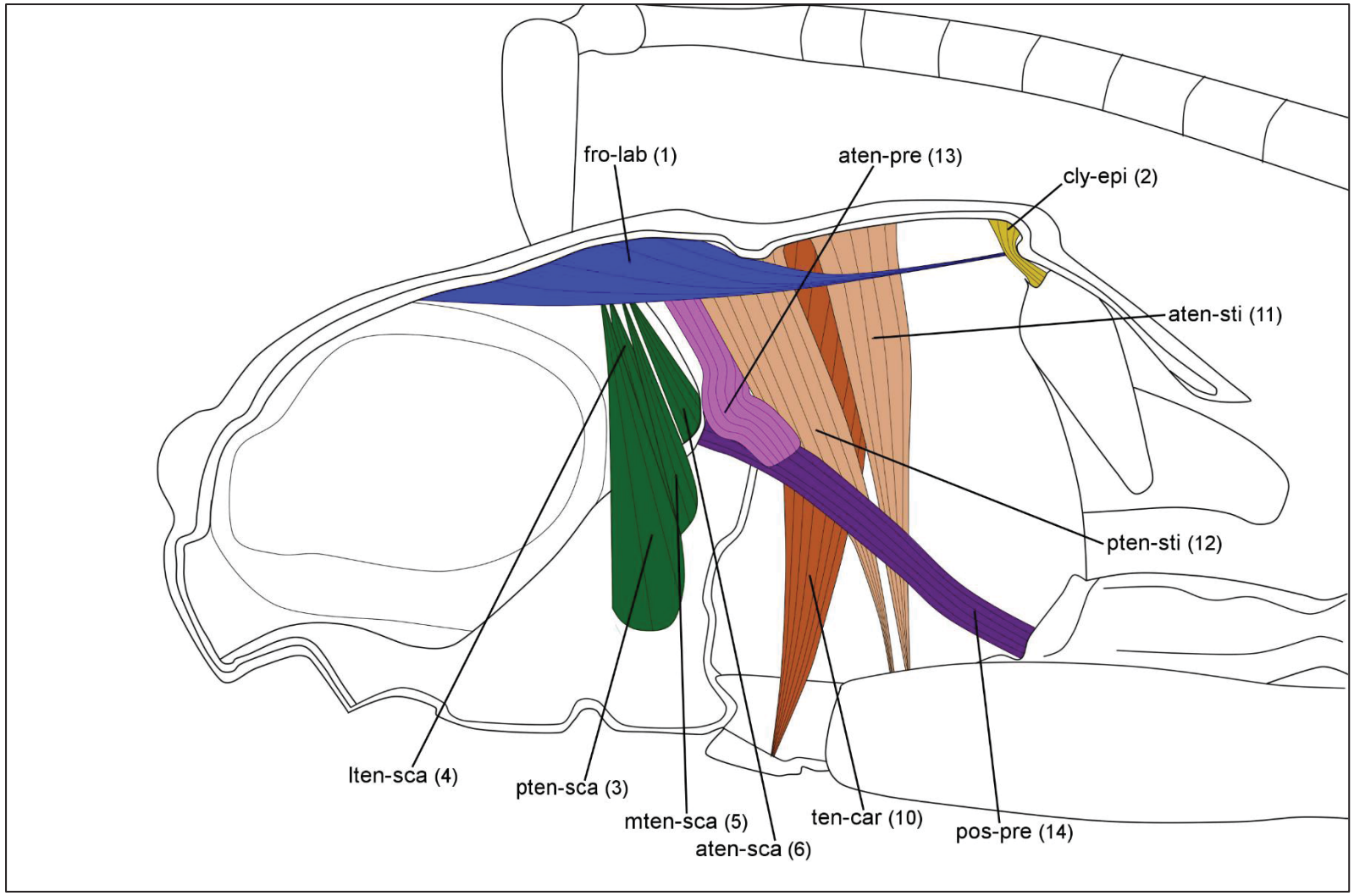

FIGURE 80. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAPEDIA DIVERSIPES* KLUG, 1810 (APIDAE: APINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

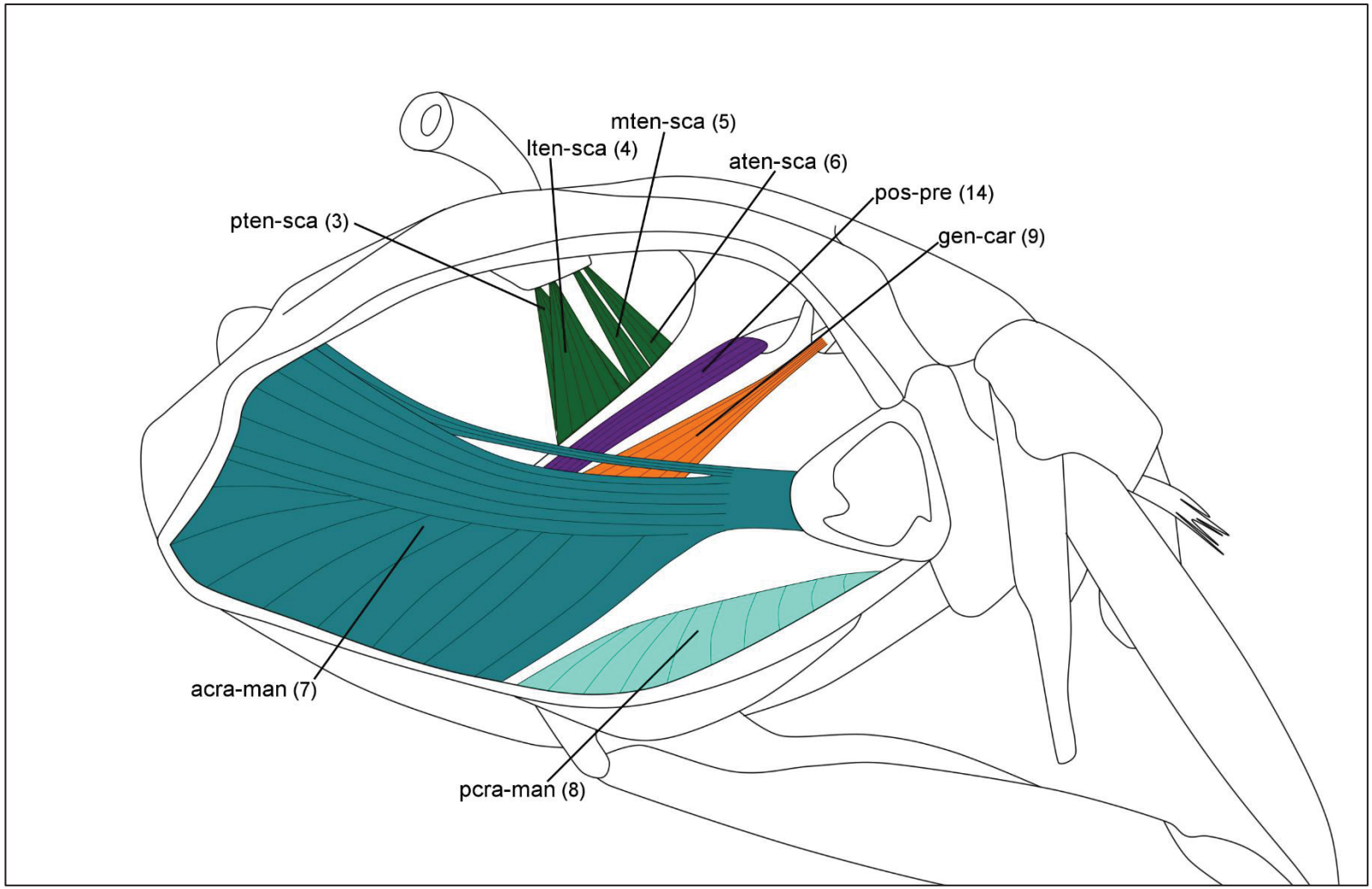

FIGURE 81. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CERATINA (RHYSOCERATINA) STILBONOTA* MOURE, 1941 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

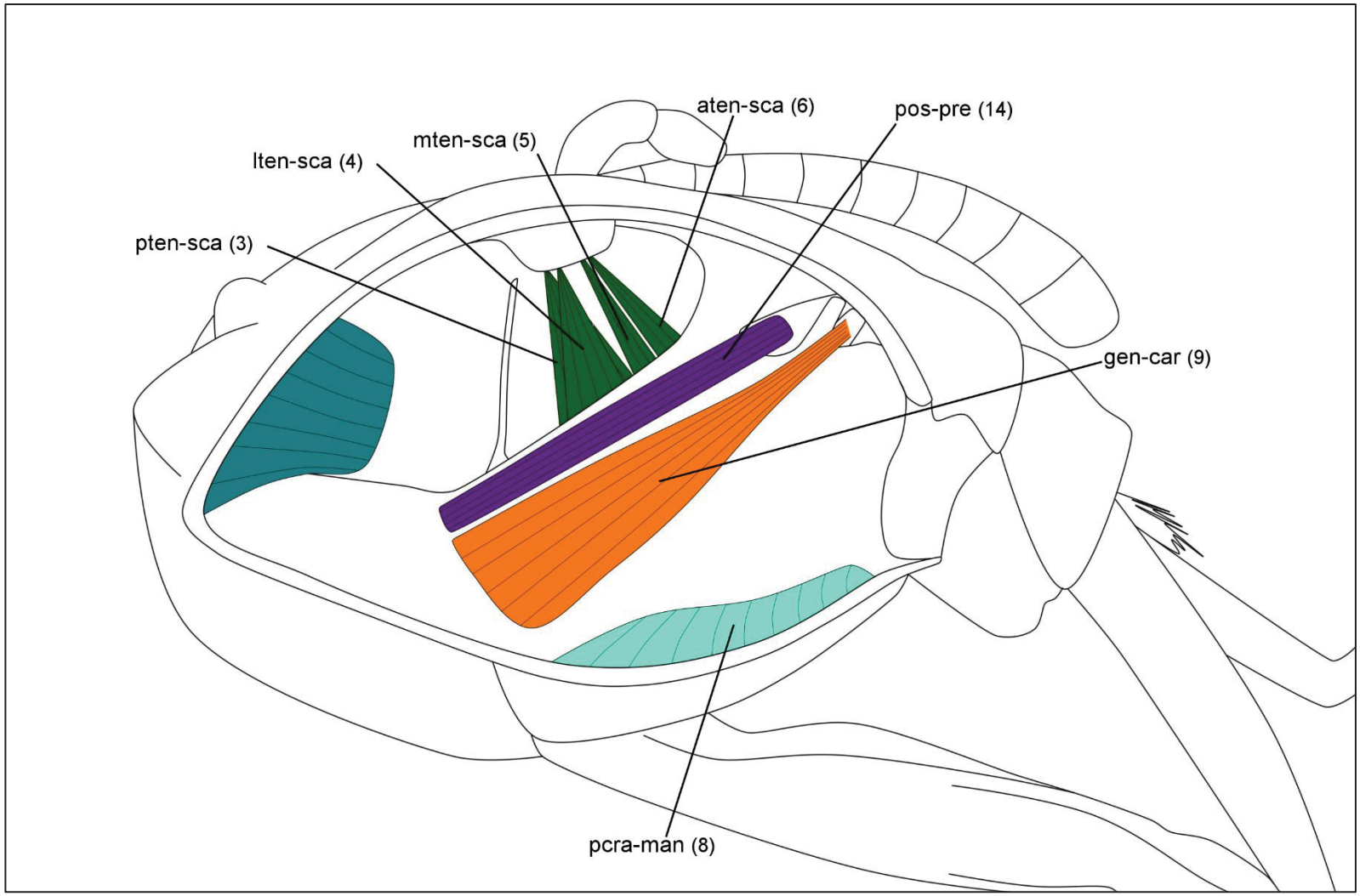

FIGURE 82. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CERATINA (RHYSOCERATINA) STILBONOTA* MOURE, 1941 (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

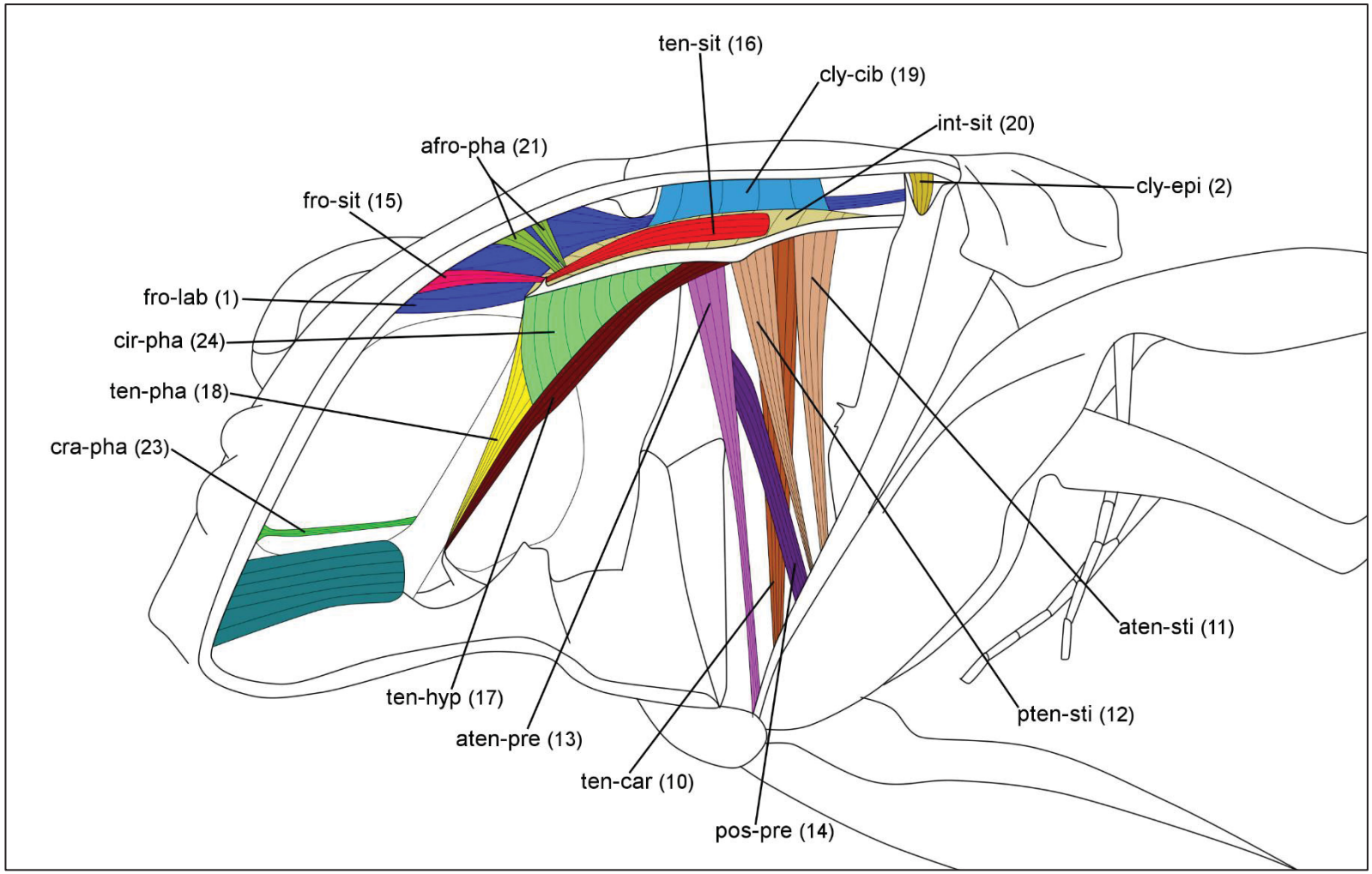

FIGURE 83. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *CERATINA (RHYSOCERATINA) STILBONOTA* MOURE, 1941 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

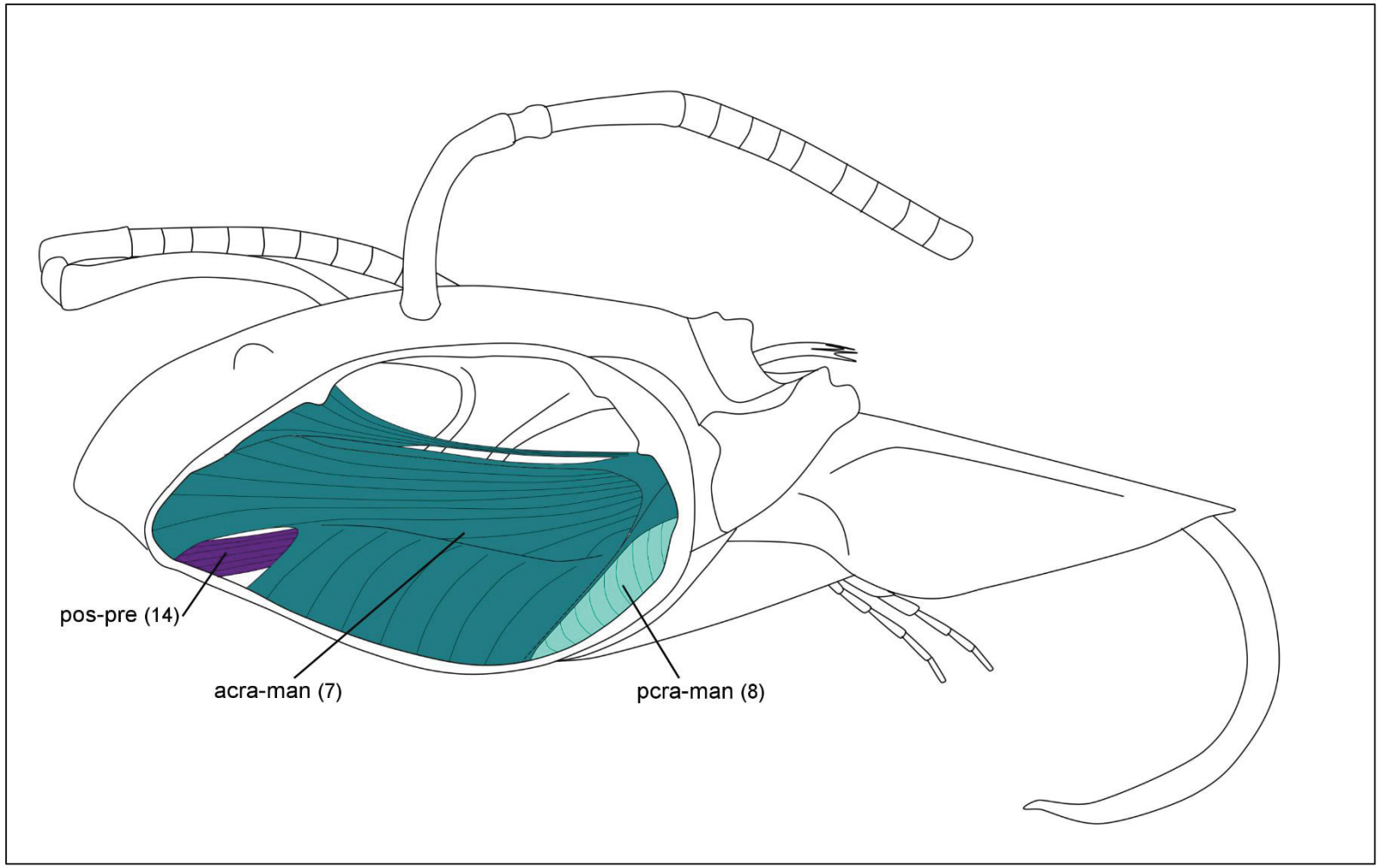

FIGURE 84. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *XYLOCOPA (NEOXYLOCOPA) AUGUSTI* LEPELETIER, 1841 (APIDAE: APINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

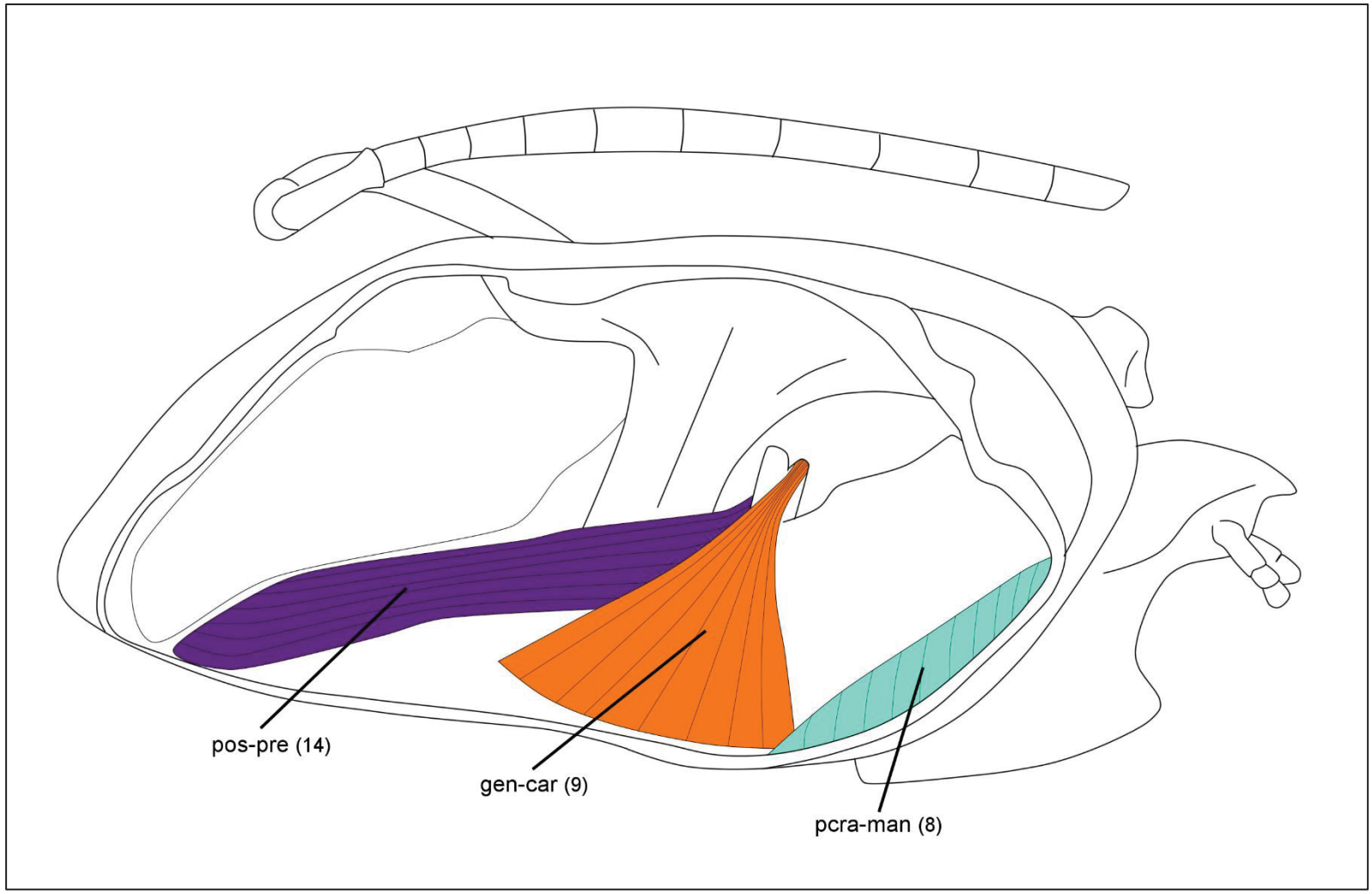

FIGURE 85. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *XYLOCOPA (NEOXYLOCOPA) AUGUSTI* LEPELETIER, 1841 (APIDAE: APINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

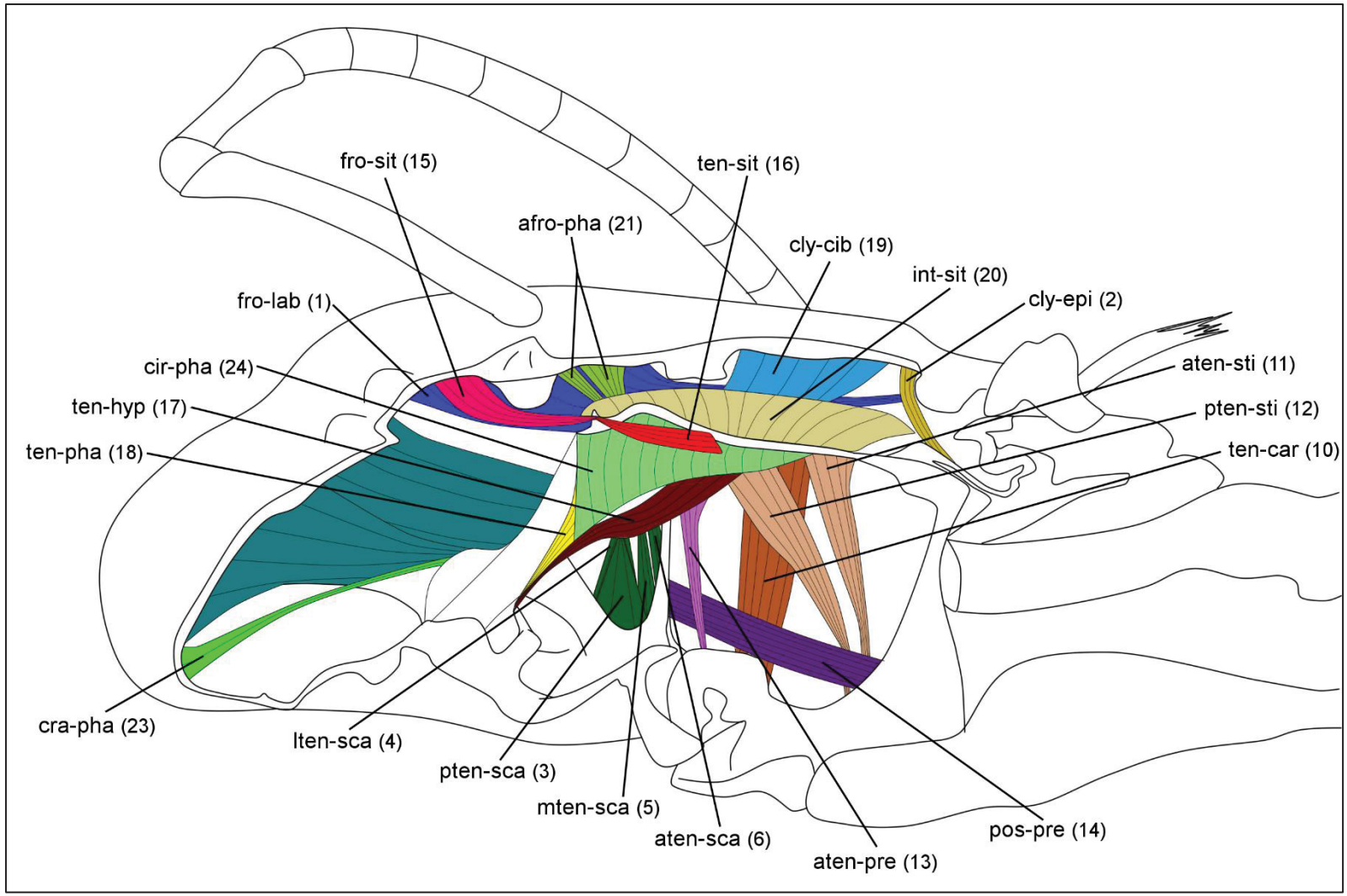

FIGURE 86. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *XYLOCOPA (NEOXYLOCOPA) AUGUSTI* LEPELETIER, 1841 (APIDAE: APINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

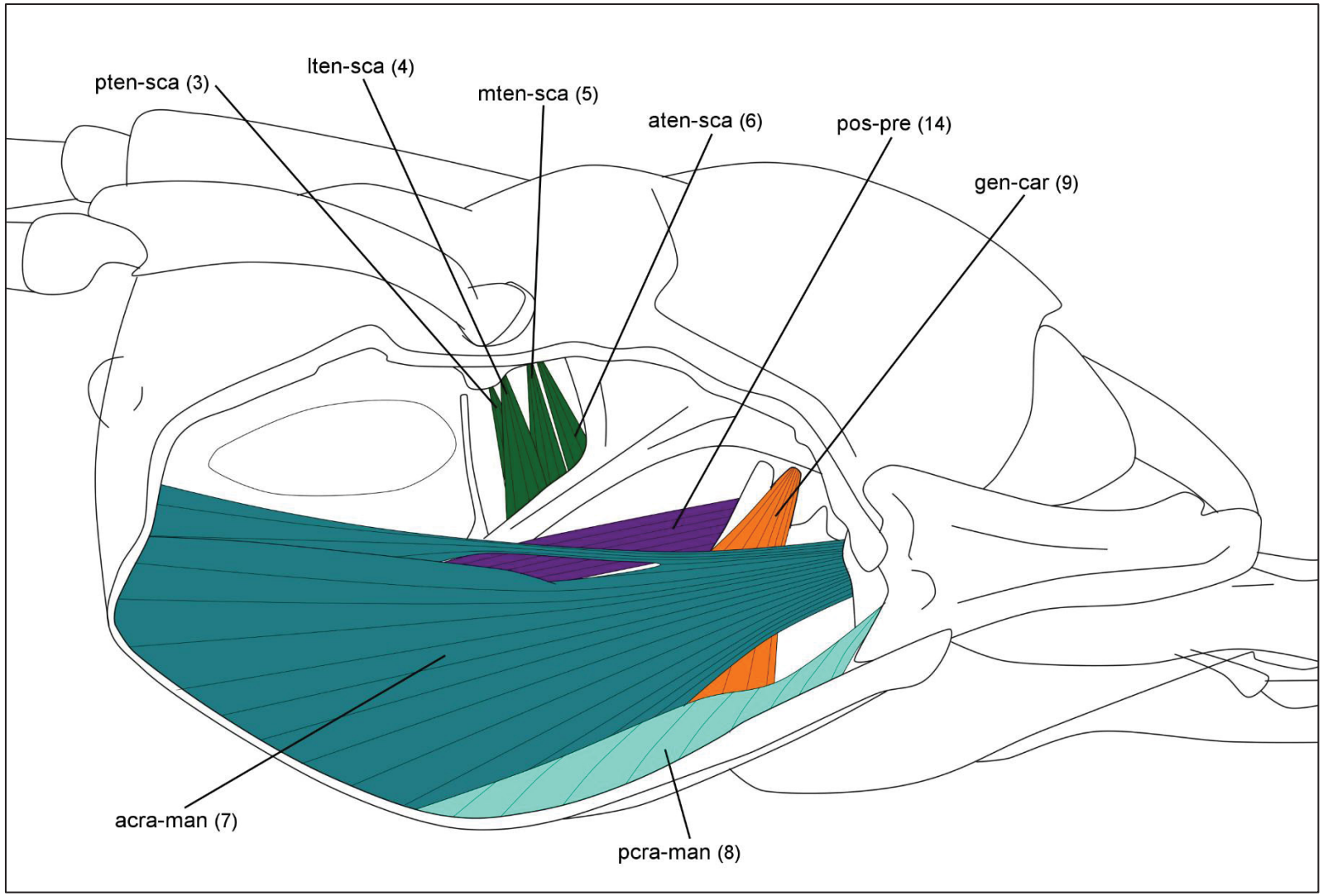

FIGURE 87. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COLLETES PETROPOLITANUS* DALLA TORRE, 1896 (APIDAE: COLLETINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

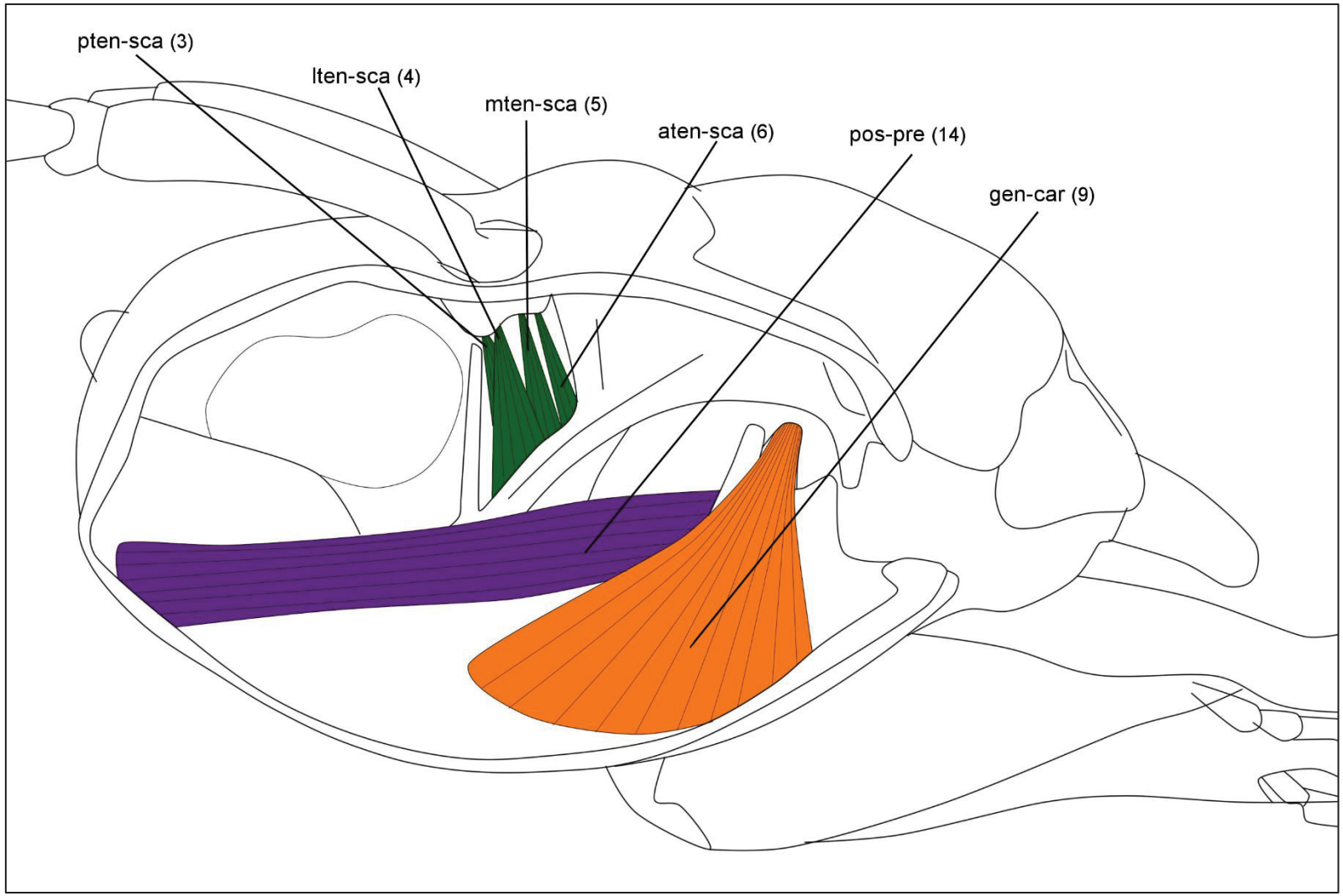

FIGURE 88. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COLLETES PETROPOLITANUS* DALLA TORRE, 1896 (APIDAE: COLLETINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

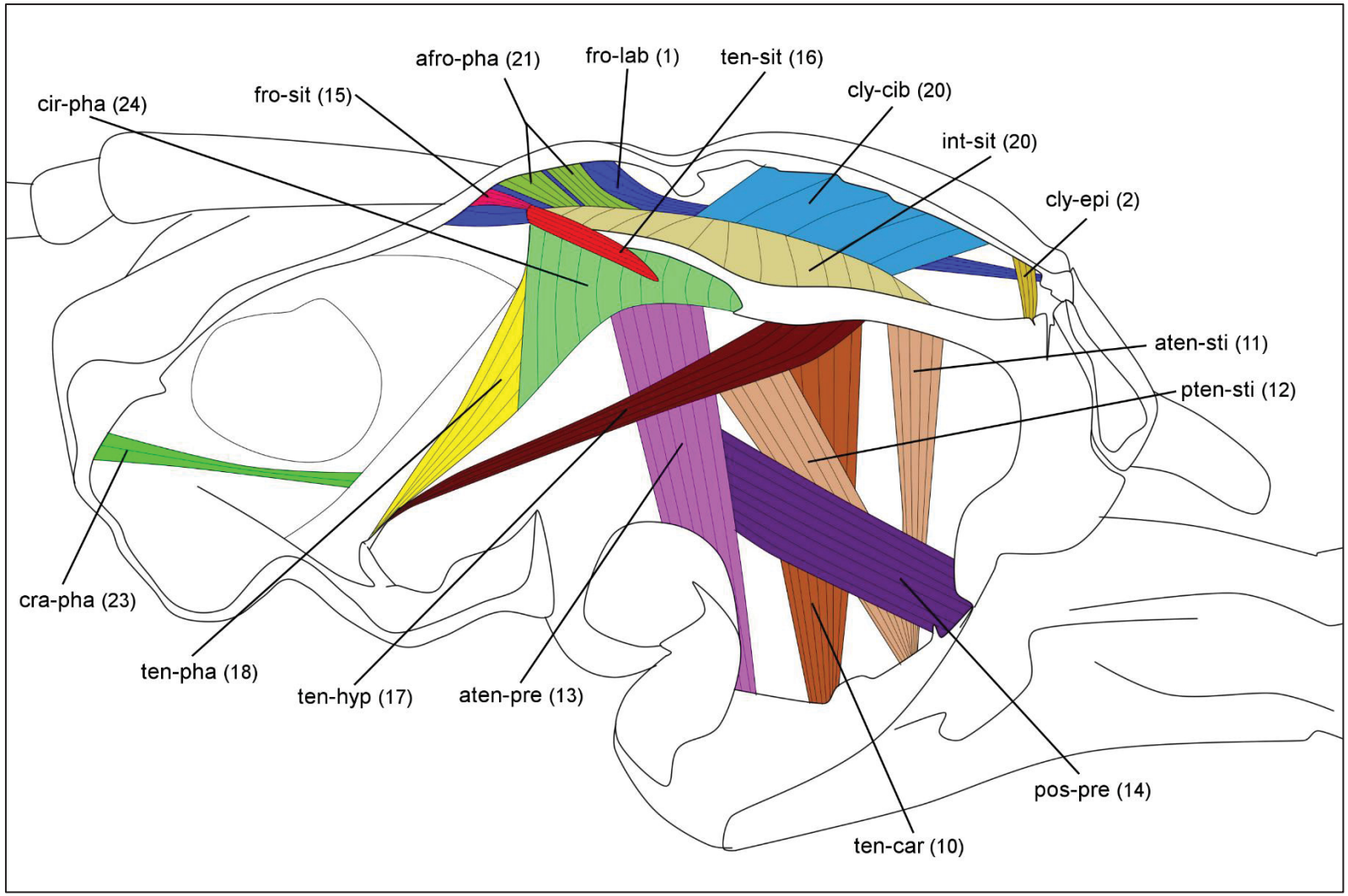

FIGURE 89. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COLLETES PETROPOLITANUS* DALLA TORRE, 1896 (APIDAE: COLLETINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

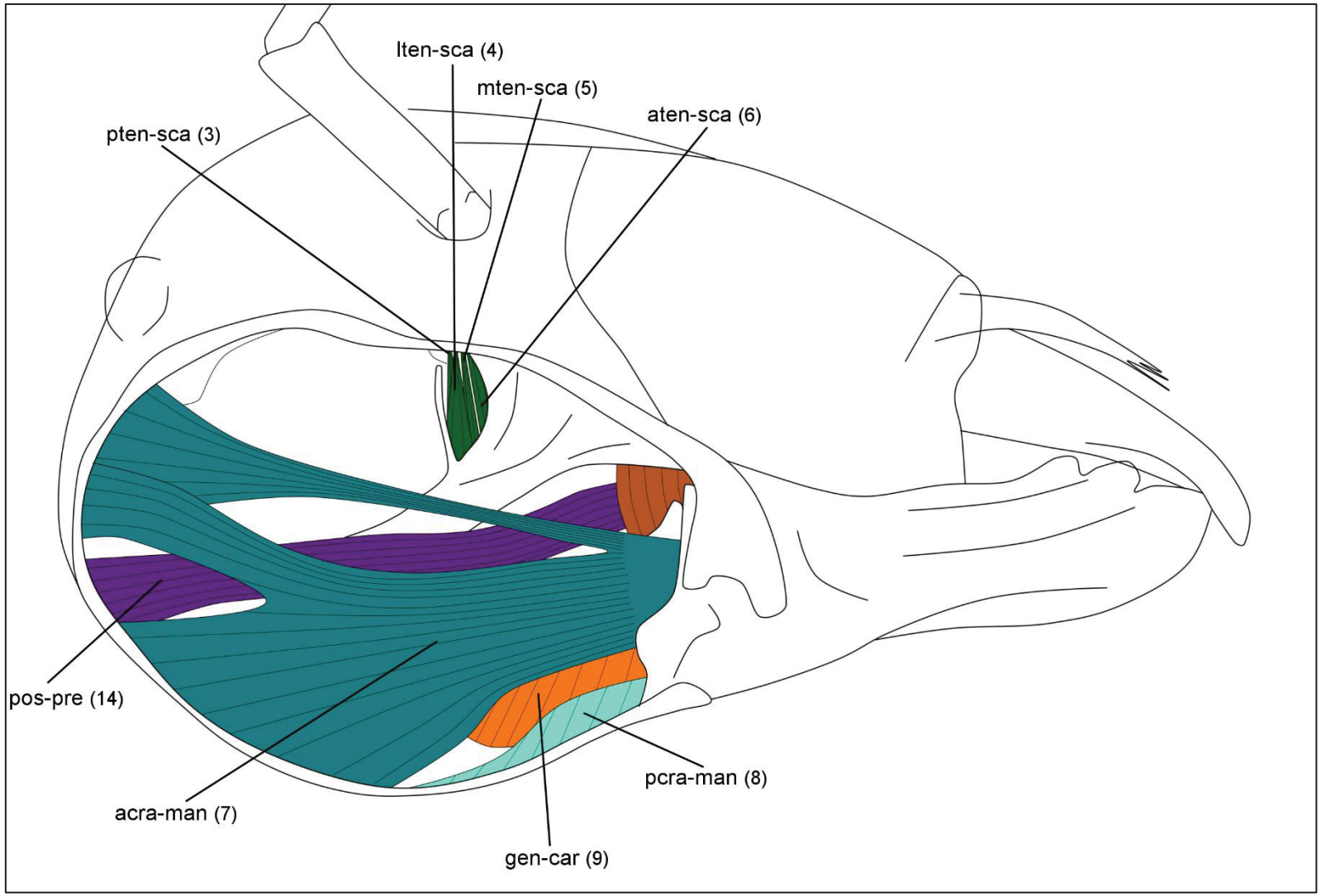

FIGURE 90. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PTILOGLOSSA* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

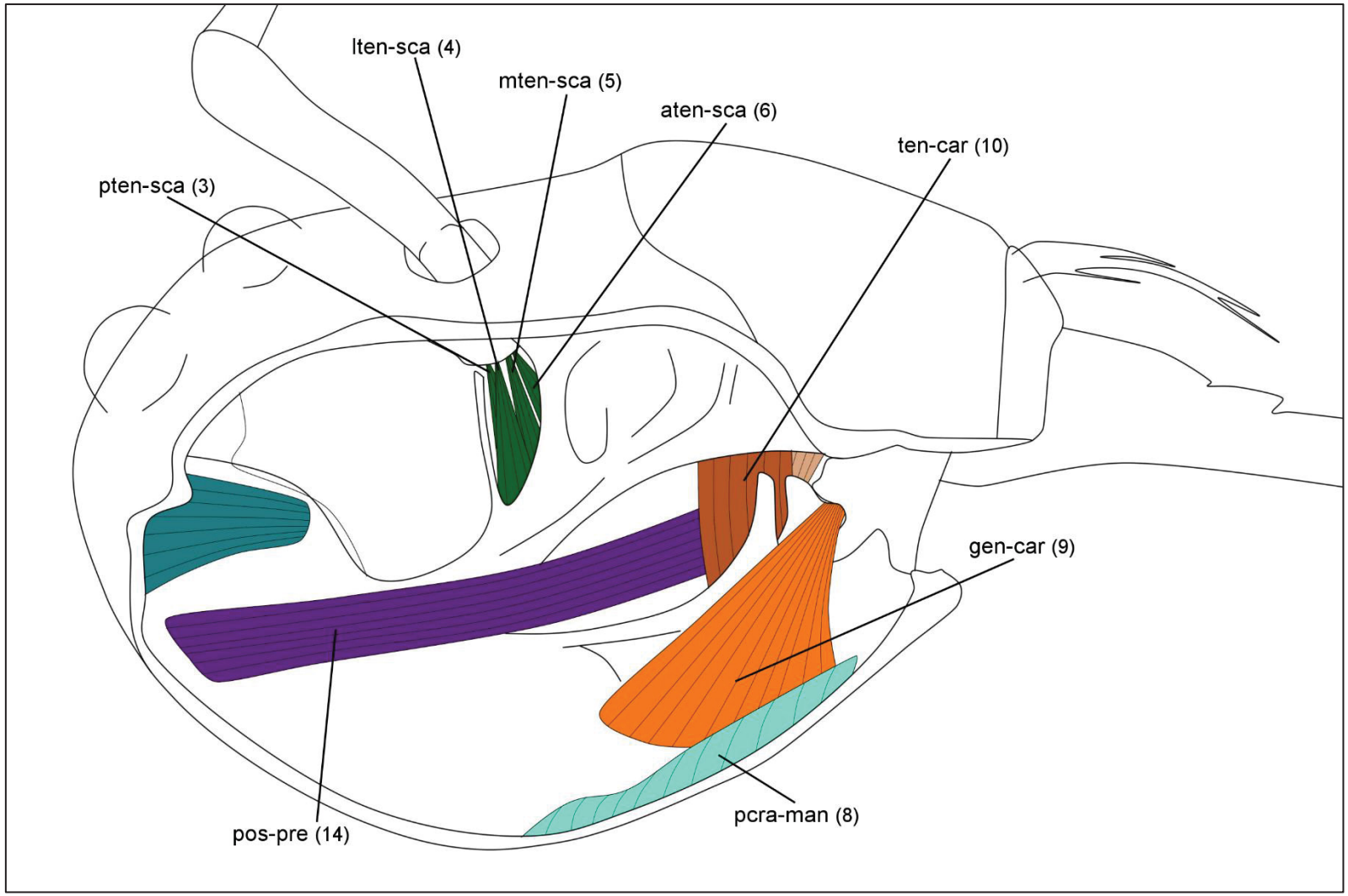

FIGURE 91. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PTILOGLOSSA* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

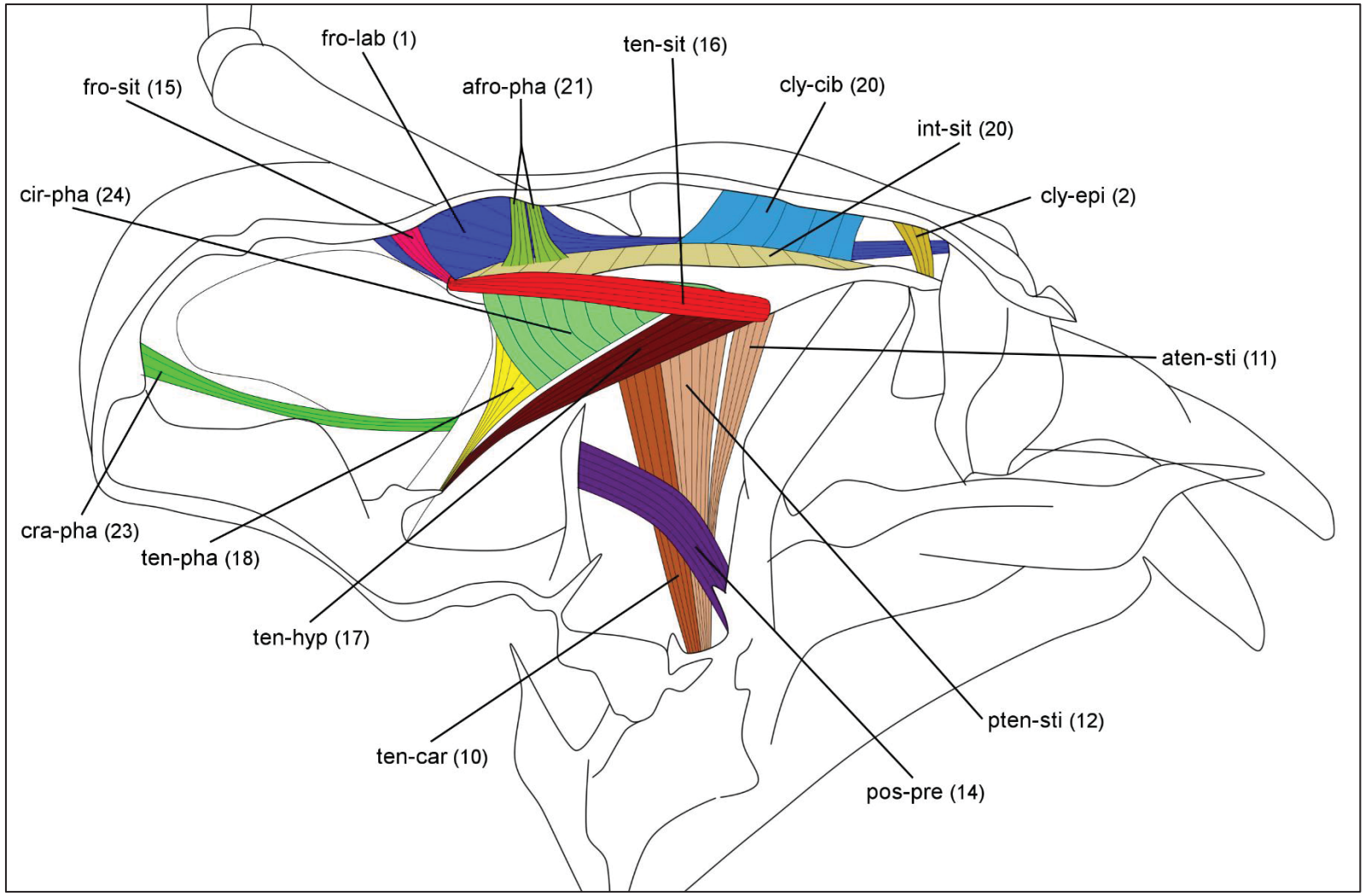

FIGURE 92. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PTILOGLOSSA* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

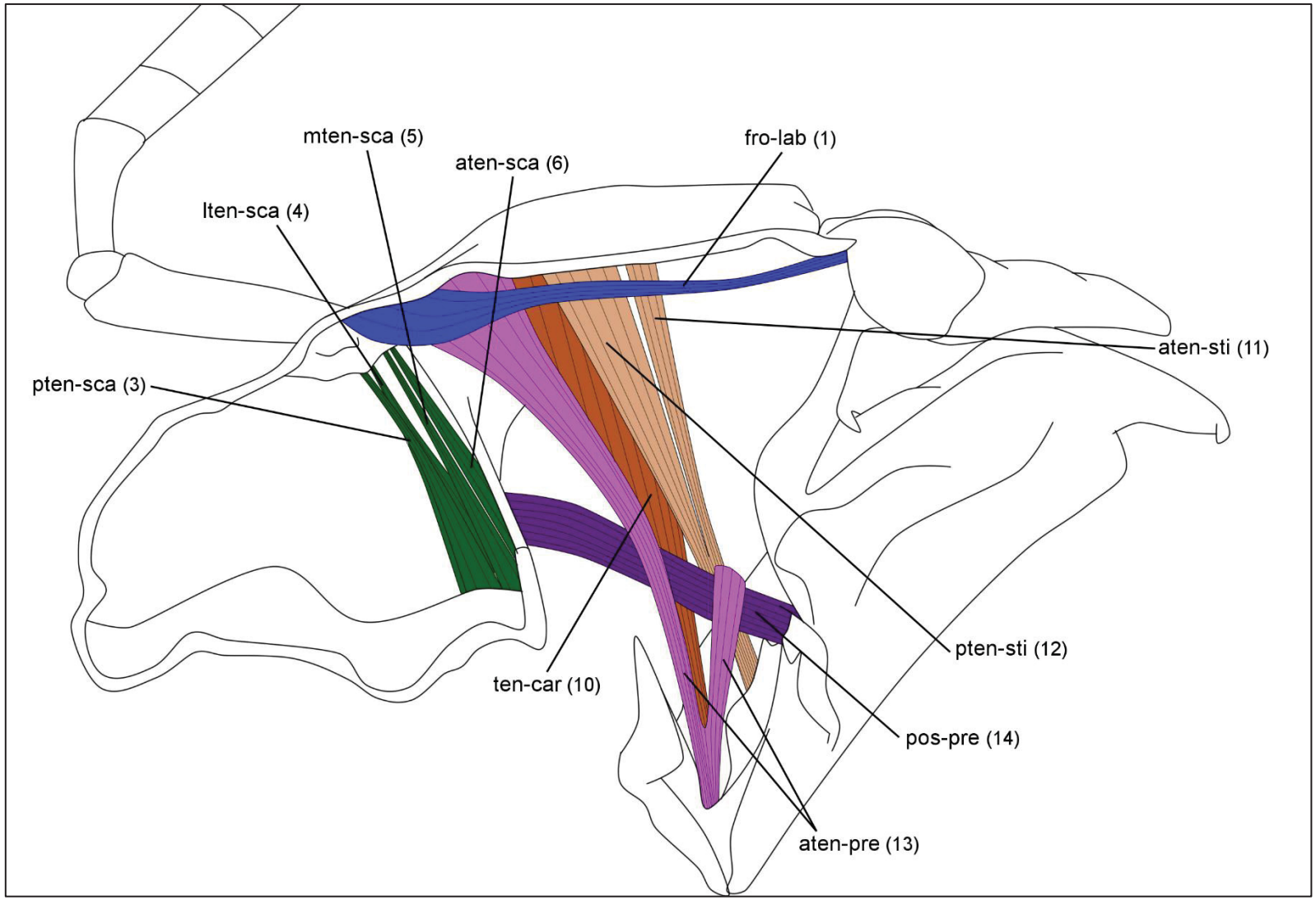

FIGURE 93. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PTILOGLOSSA* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

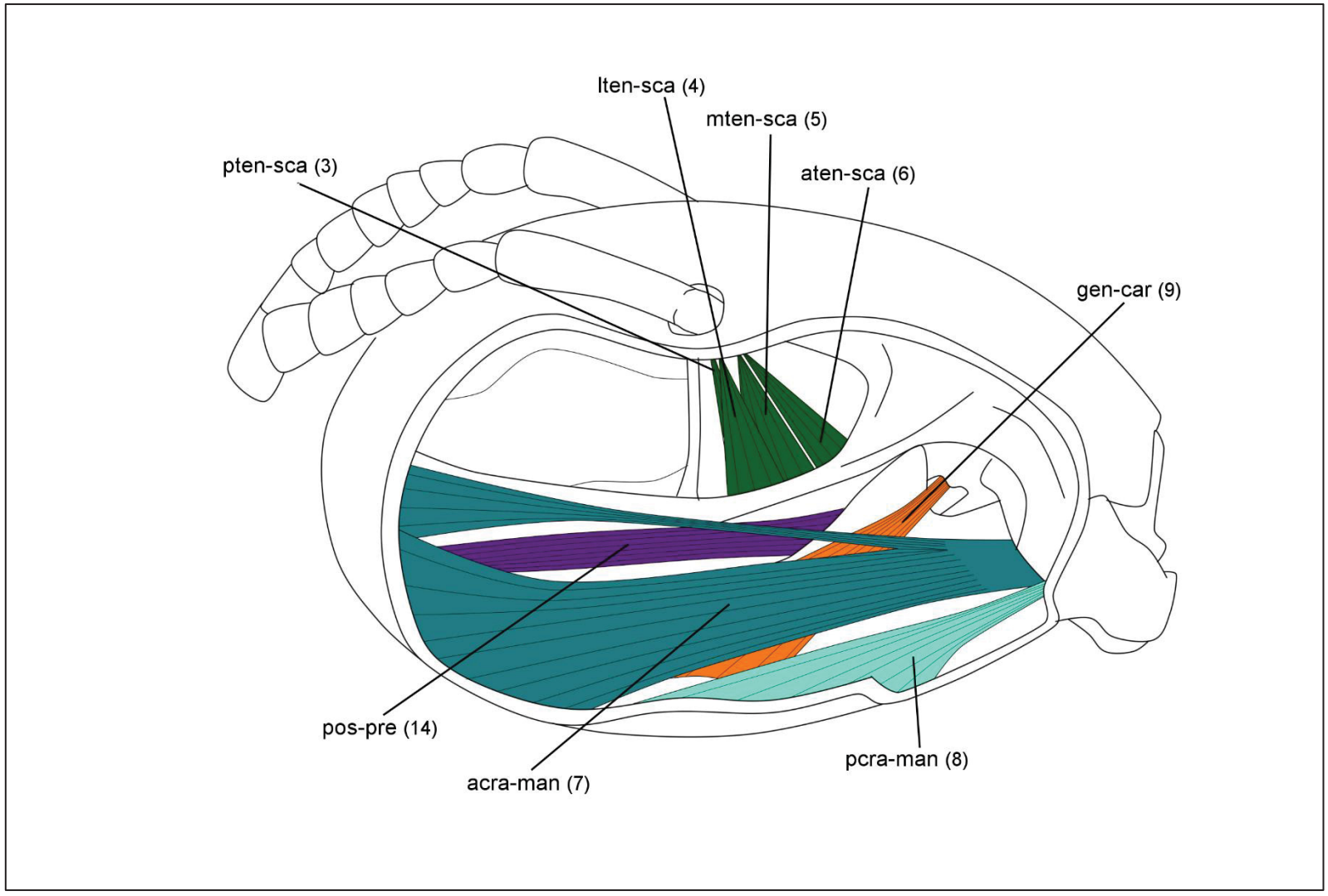

FIGURE 94. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *HYLAEUS* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

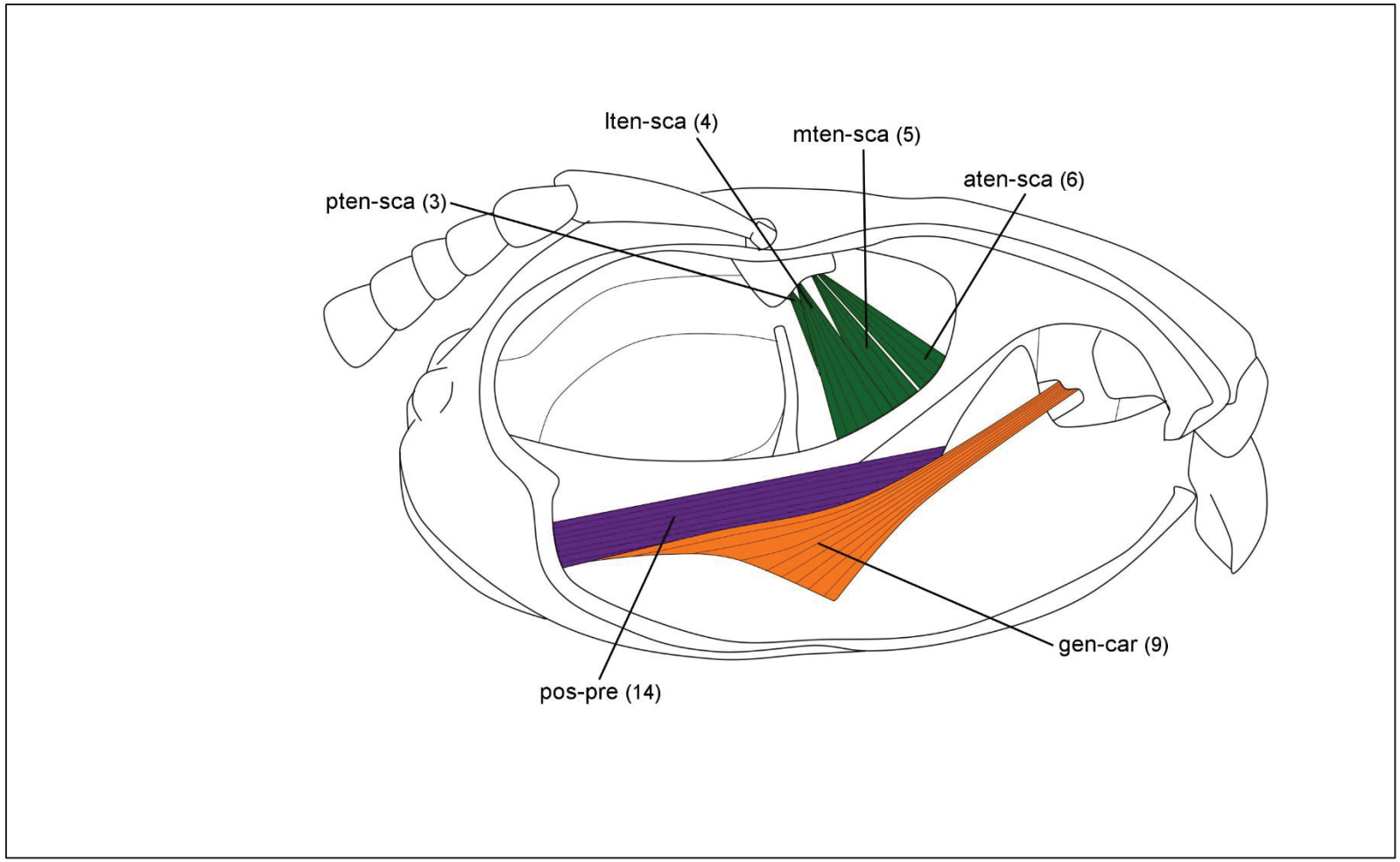

FIGURE 95. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *HYLAEUS* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

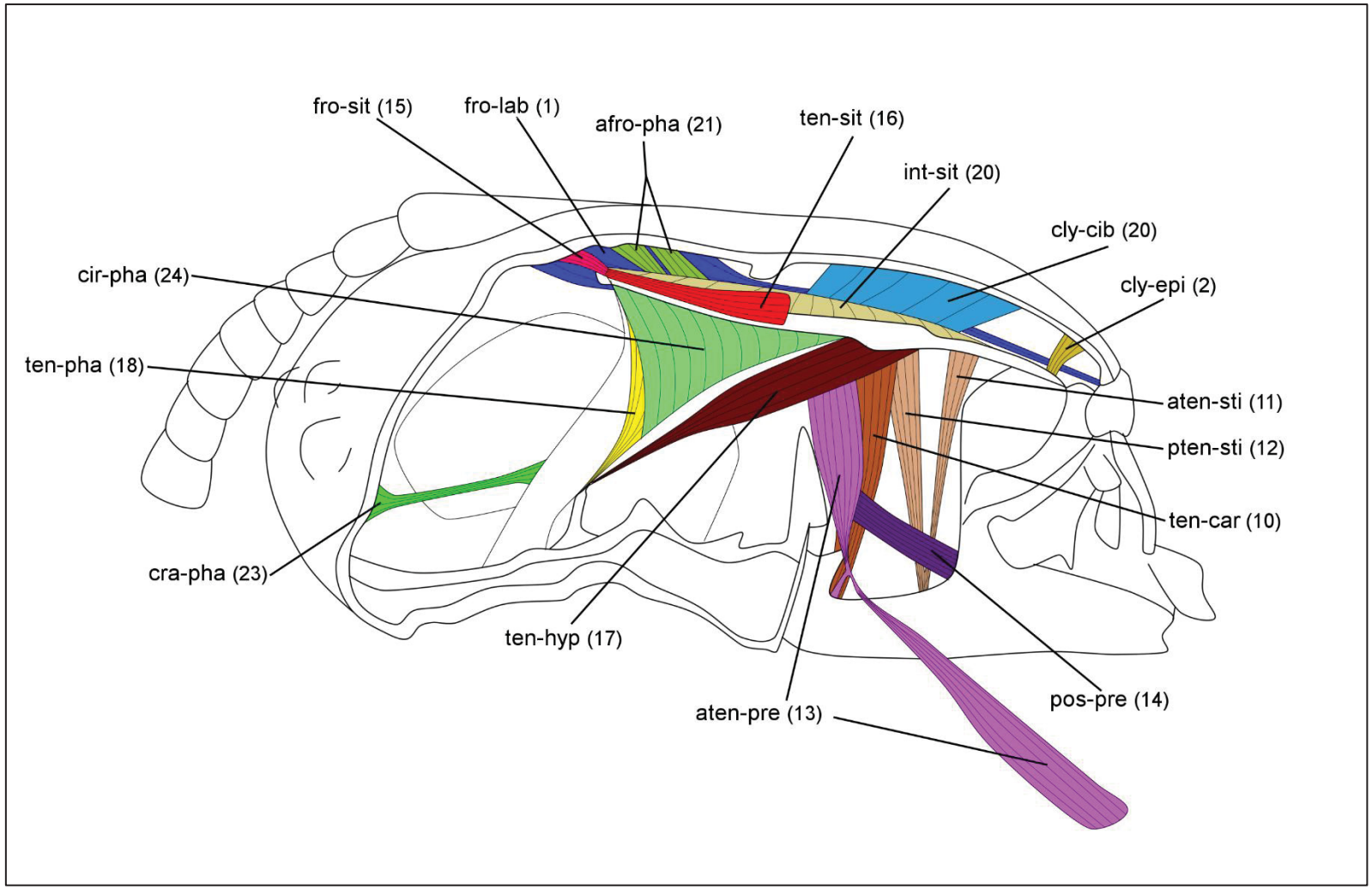

FIGURE 96. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *HYLAEUS* SP. (APIDAE: COLLETINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

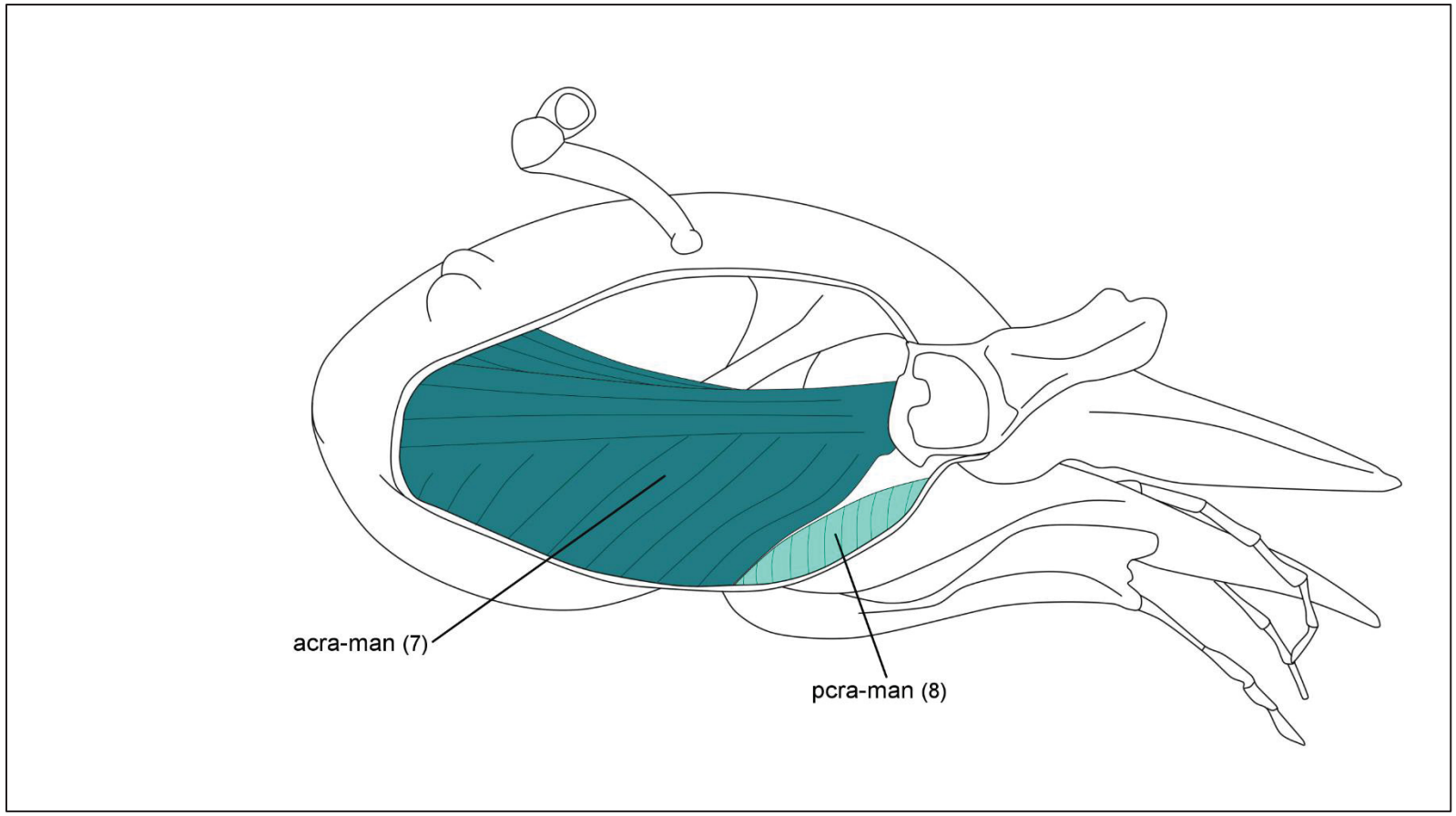

FIGURE 97. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAGLOSSULA ANTHRACINA* (MICHENER, 1989) (APIDAE: COLLETINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

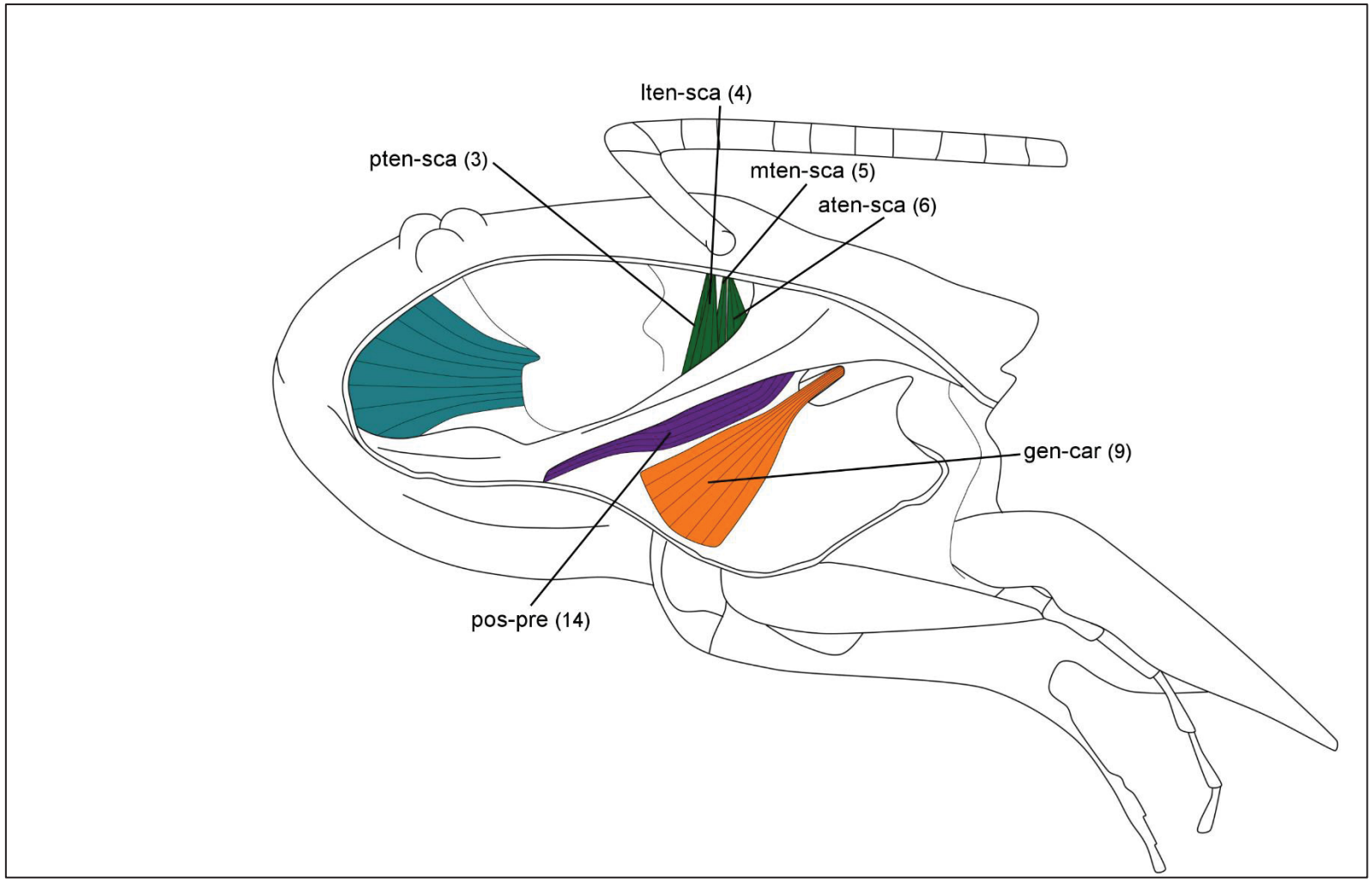

FIGURE 98. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAGLOSSULA ANTHRACINA* (MICHENER, 1989) (APIDAE: COLLETINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

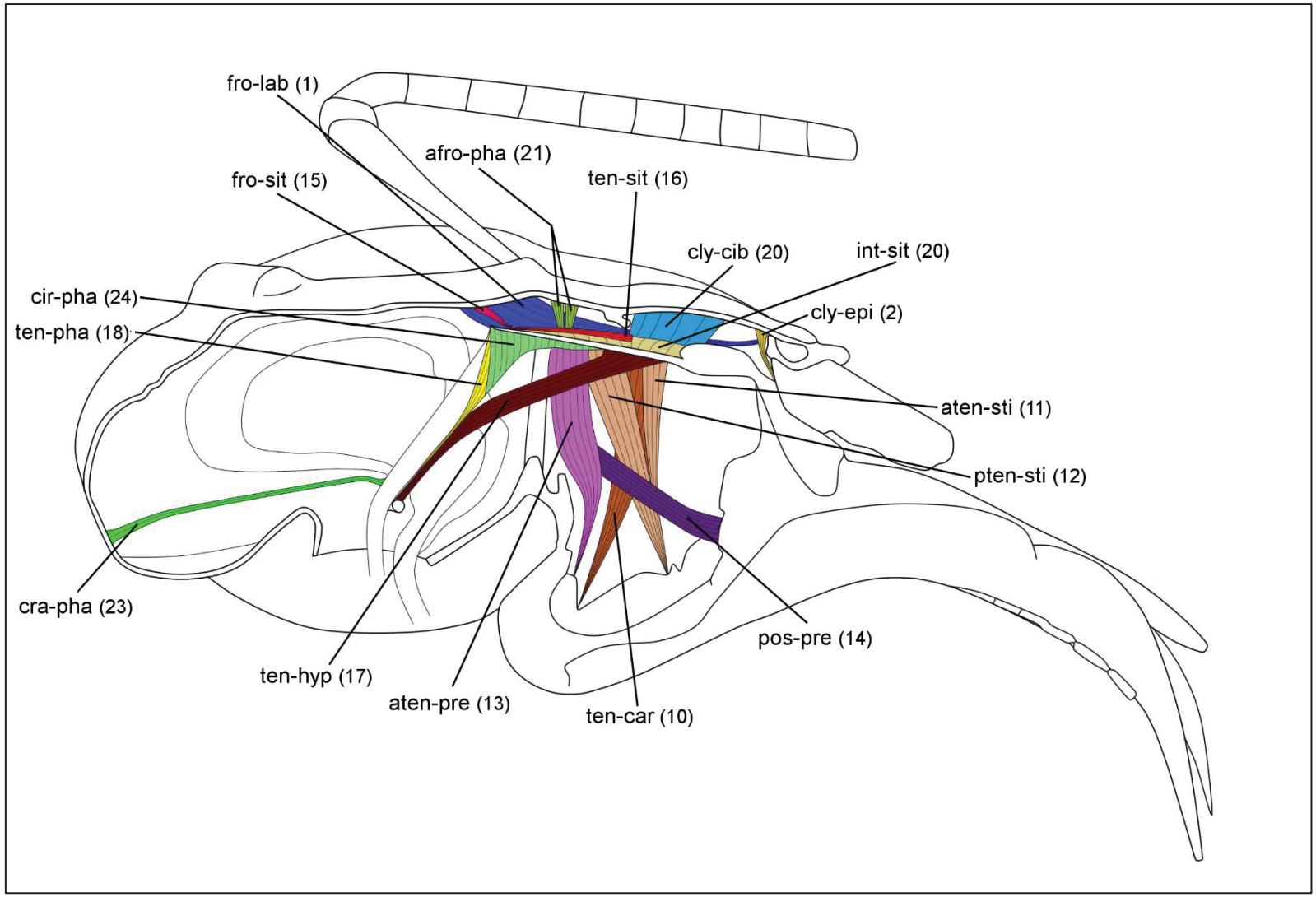

FIGURE 99. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TETRAGLOSSULA ANTHRACINA* (MICHENER, 1989) (APIDAE: COLLETINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

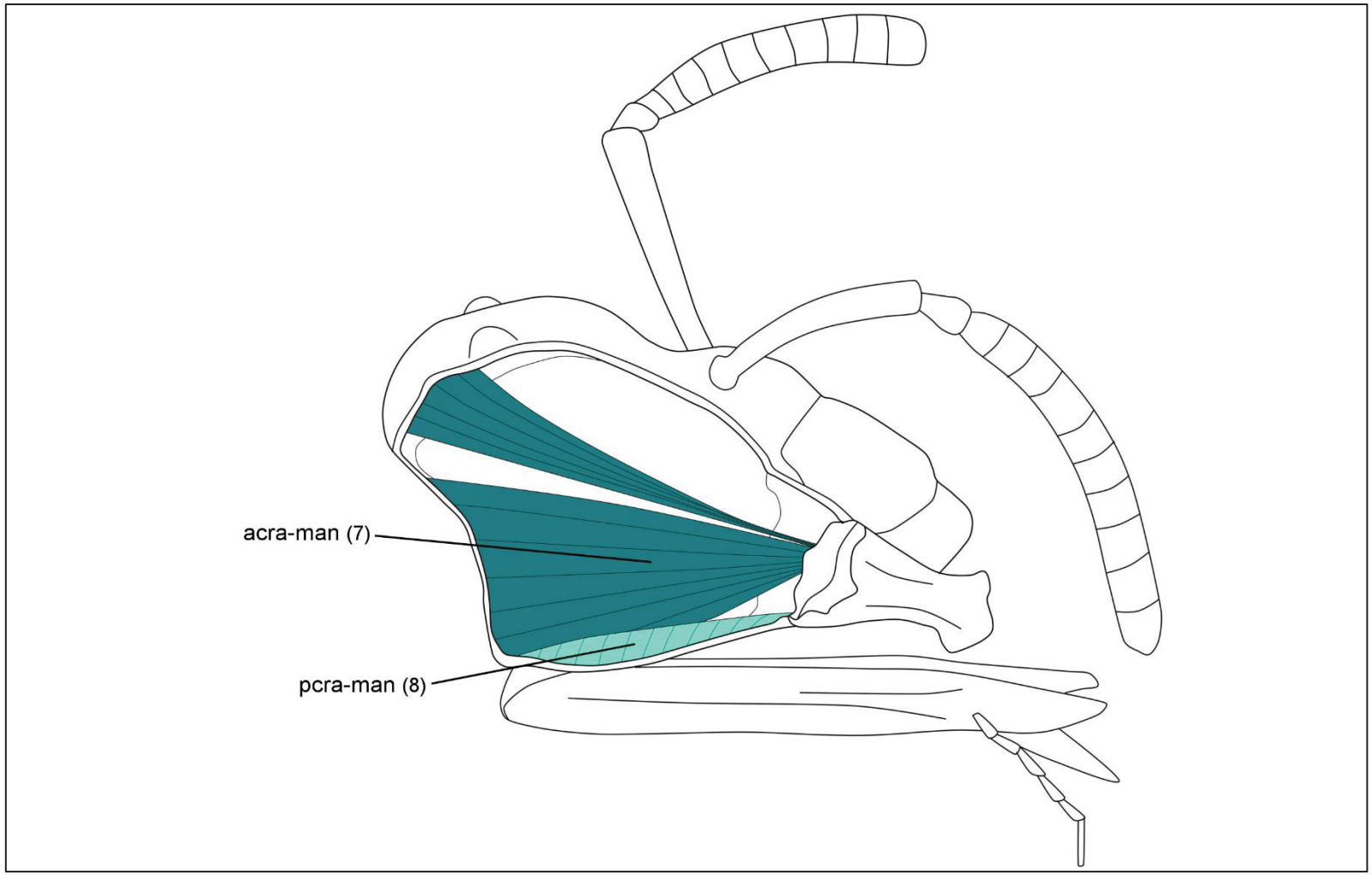

FIGURE 100. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

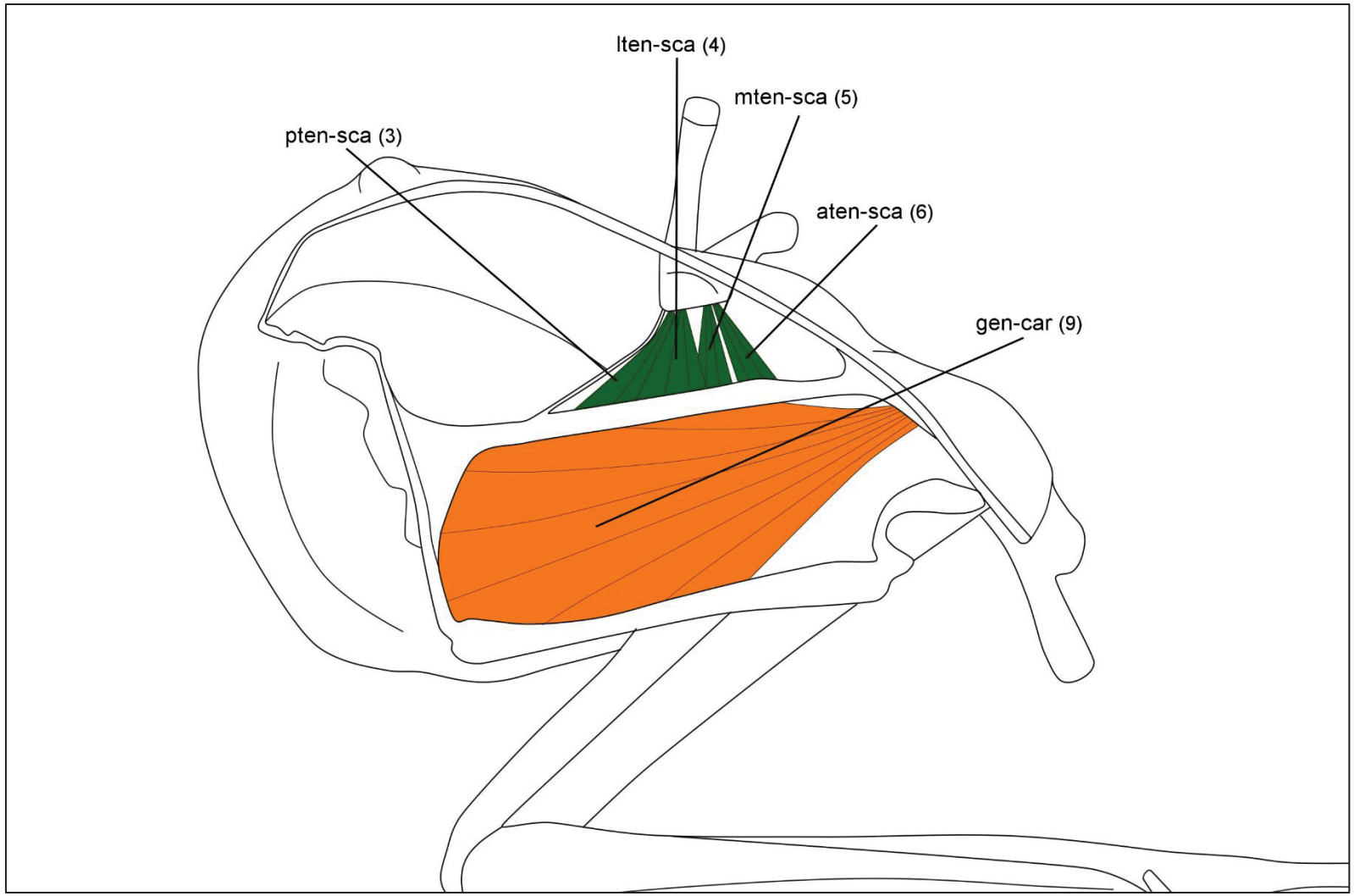

FIGURE 101. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

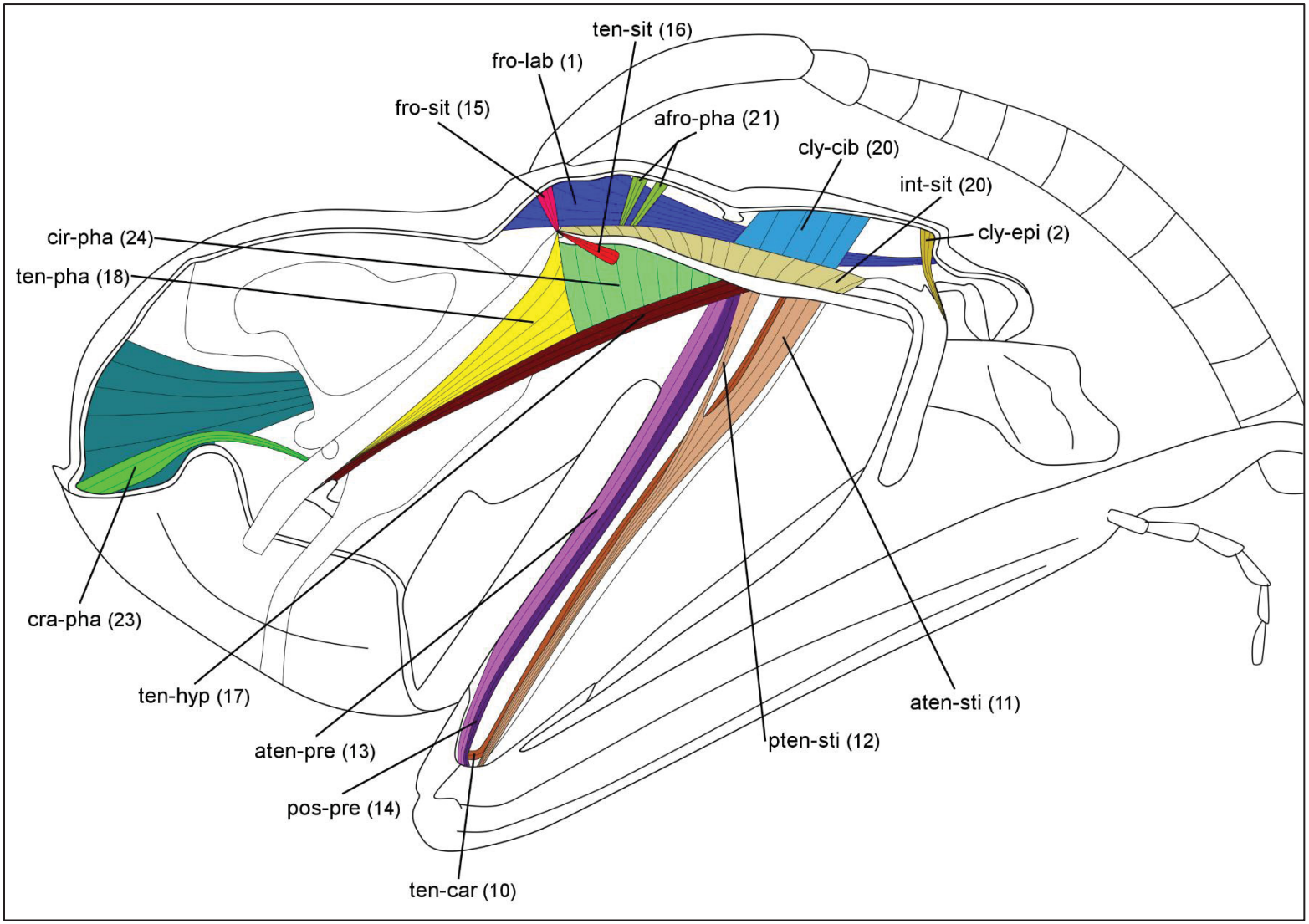

FIGURE 102. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

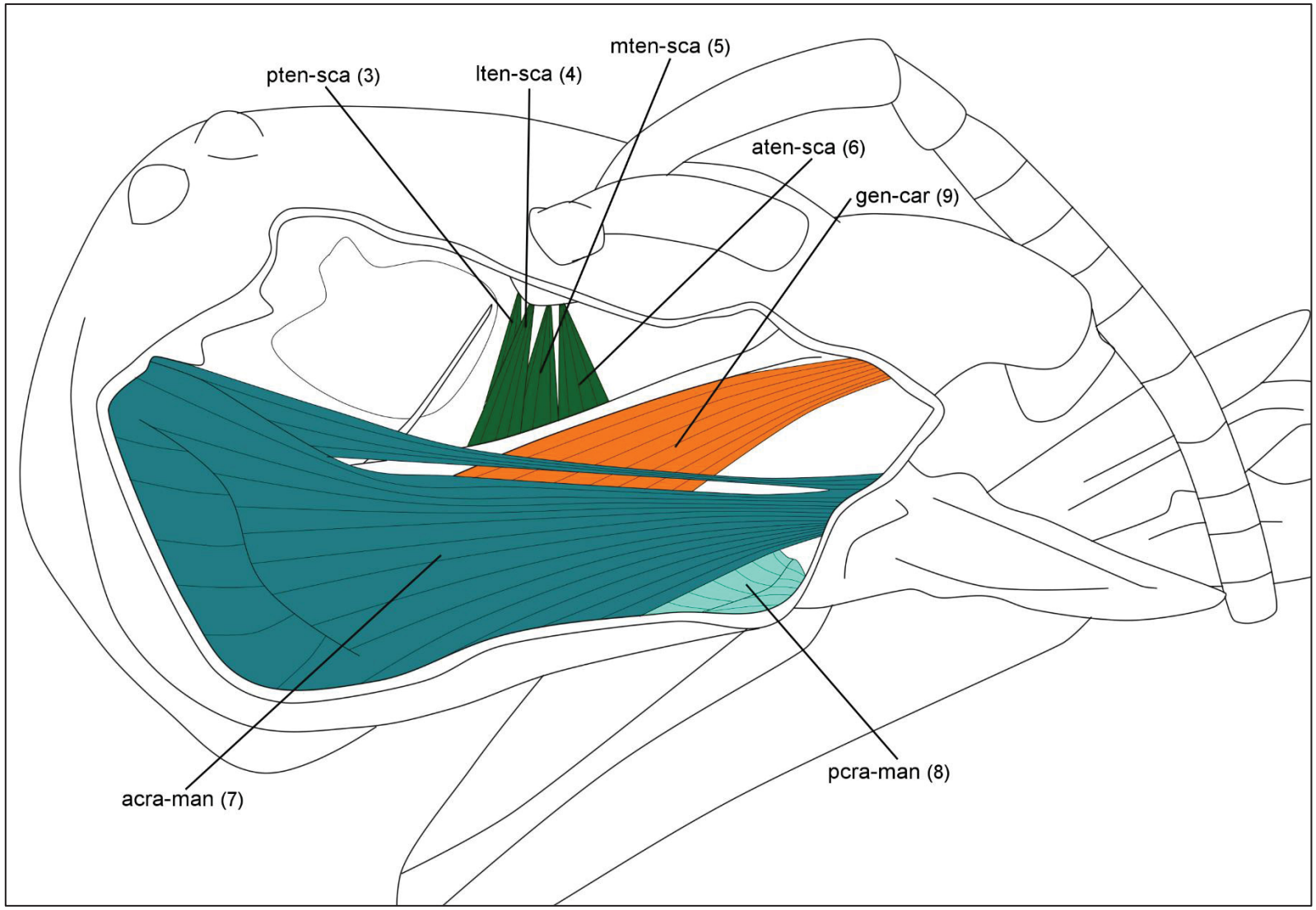

FIGURE 103. EXTRINSIC MUSCLES OF THE HEAD OF MALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

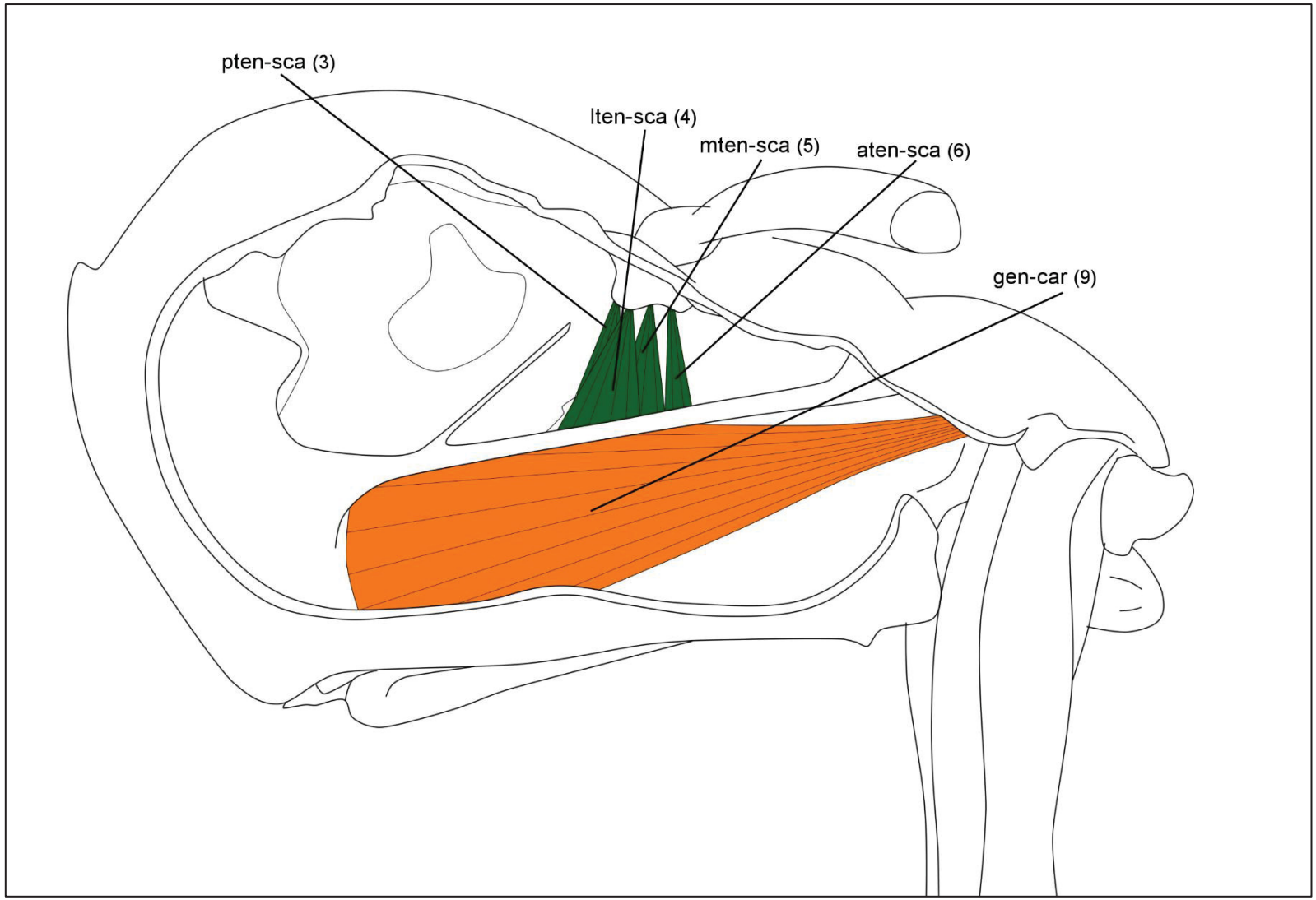

FIGURE 104. EXTRINSIC MUSCLES OF THE HEAD OF MALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

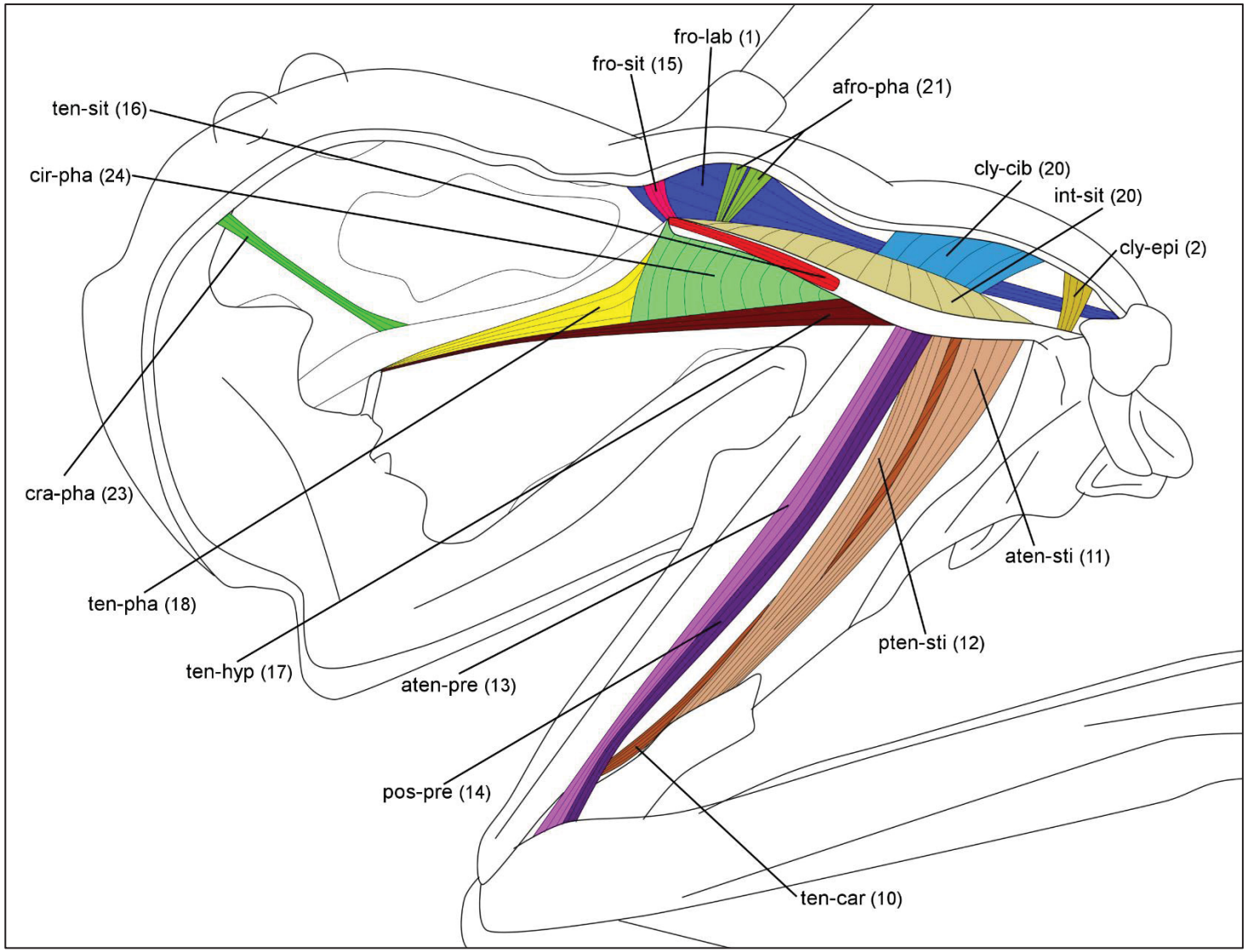

FIGURE 105. EXTRINSIC MUSCLES OF THE HEAD OF MALE *AUGOCHLORA (AUGOCHLORA) DAPHNIS* SMITH, 1853 (APIDAE: HALICTINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

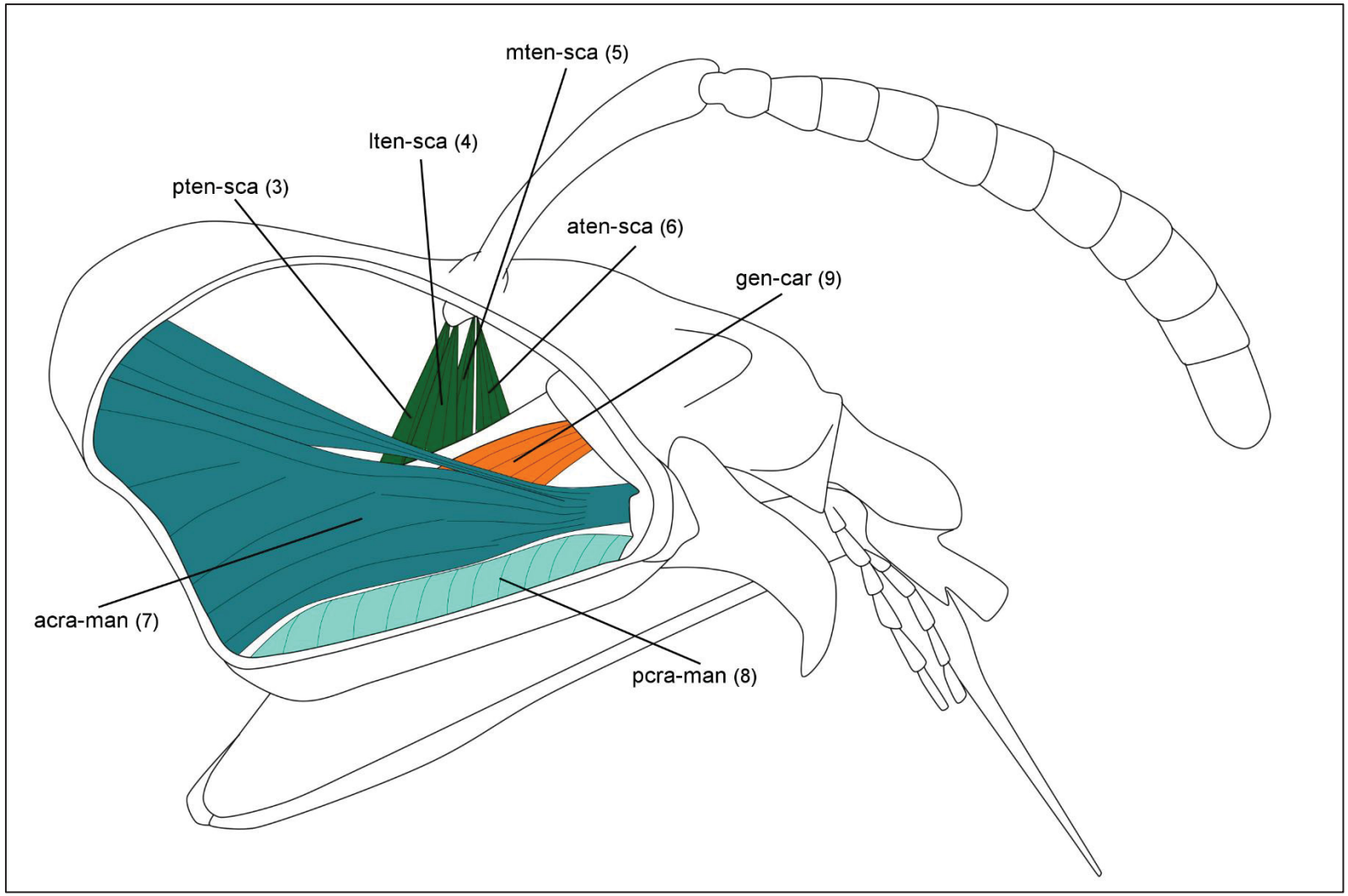

FIGURE 106. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AGAPOSTEMON (NOTAGAPOSTEMON) SEMIMELLEUS* COCKERELL, 1900 (APIDAE: HALICTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

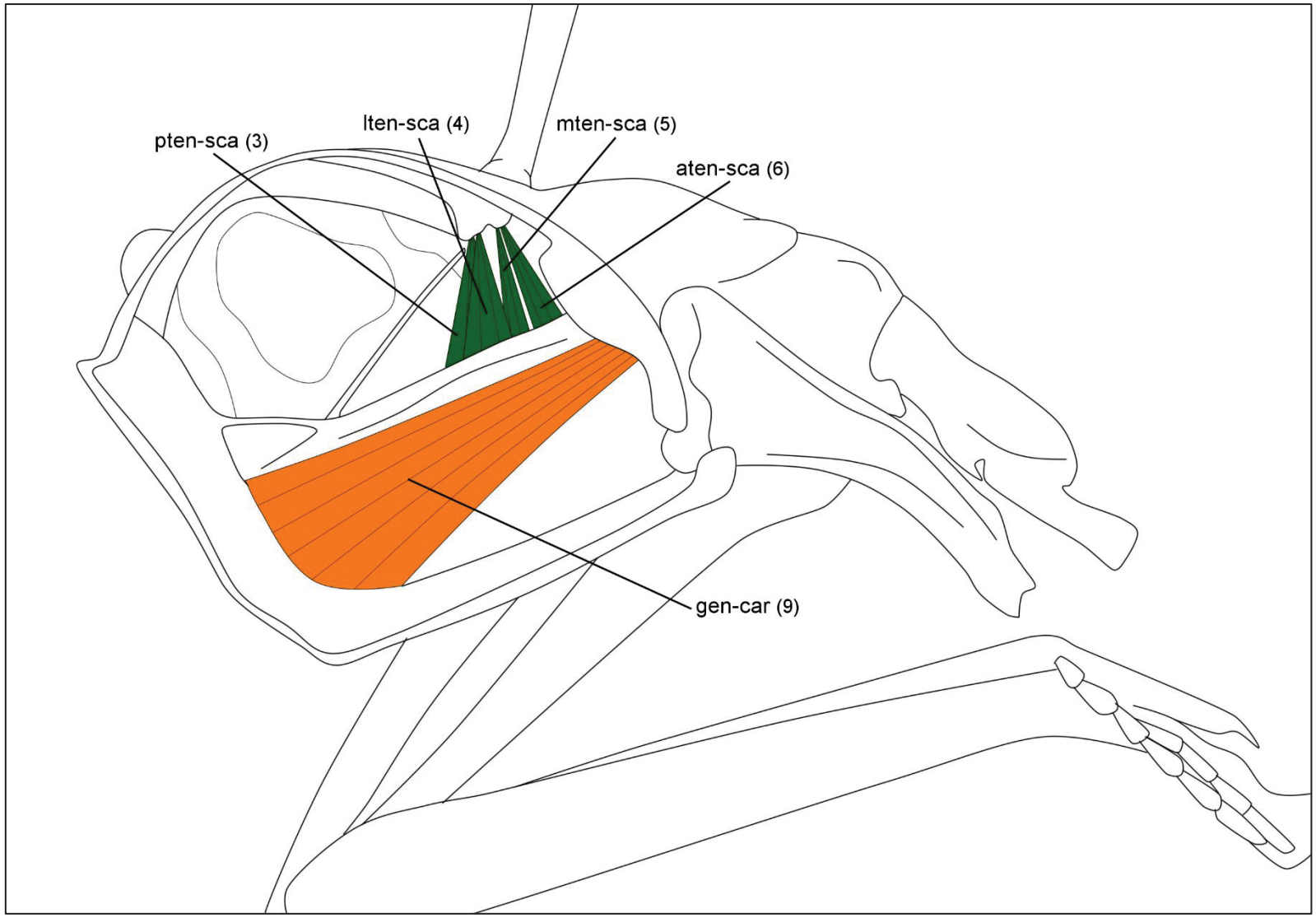

FIGURE 107. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AGAPOSTEMON (NOTAGAPOSTEMON) SEMIMELLEUS* COCKERELL, 1900 (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

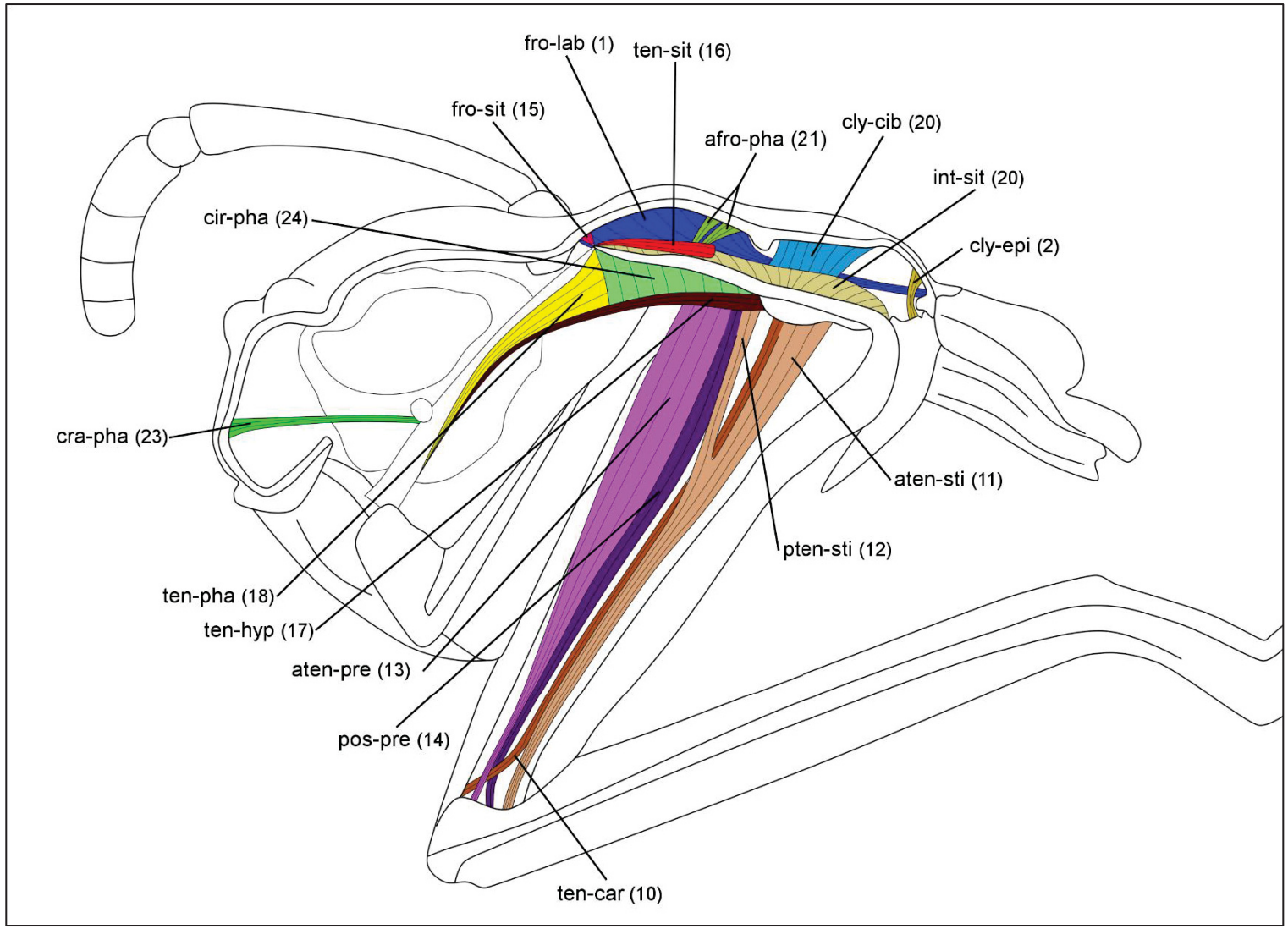

FIGURE 108. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *AGAPOSTEMON (NOTAGAPOSTEMON) SEMIMELLEUS* COCKERELL, 1900 (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

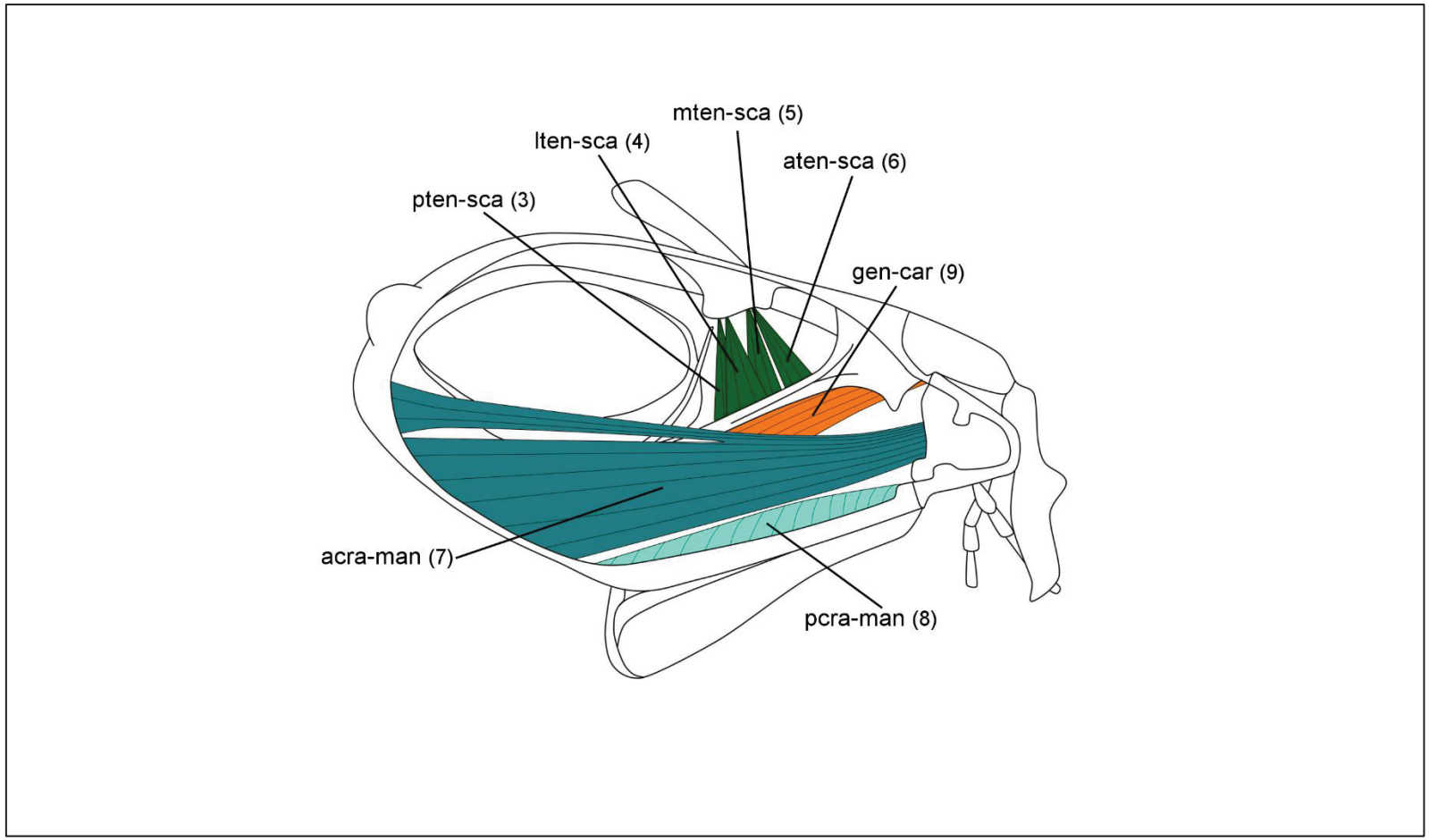

FIGURE 109. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DIALICTUS OPACUS* (MOURE, 1940) (APIDAE: HALICTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

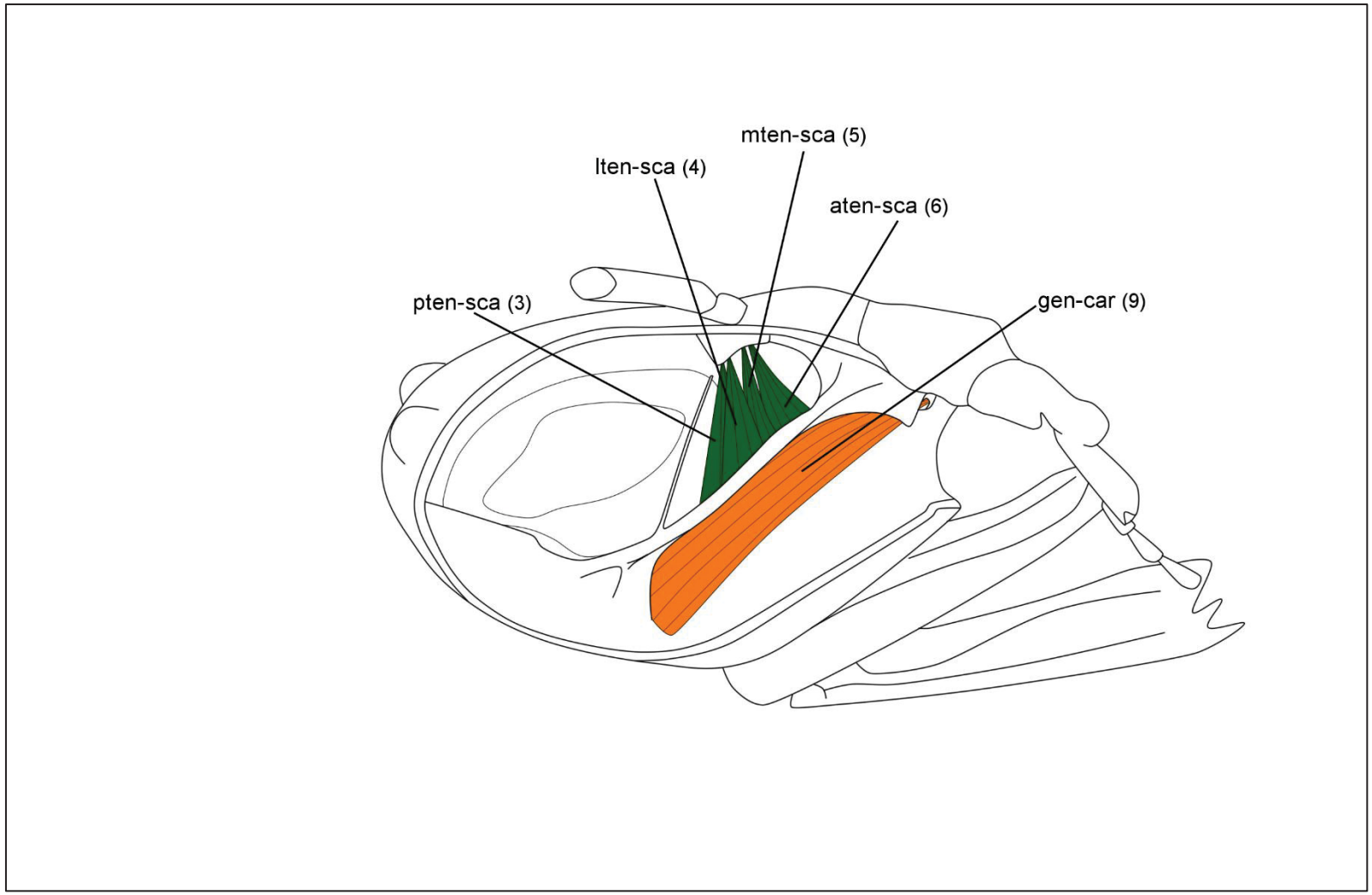

FIGURE 110. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DIALICTUS OPACUS* (MOURE, 1940) (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

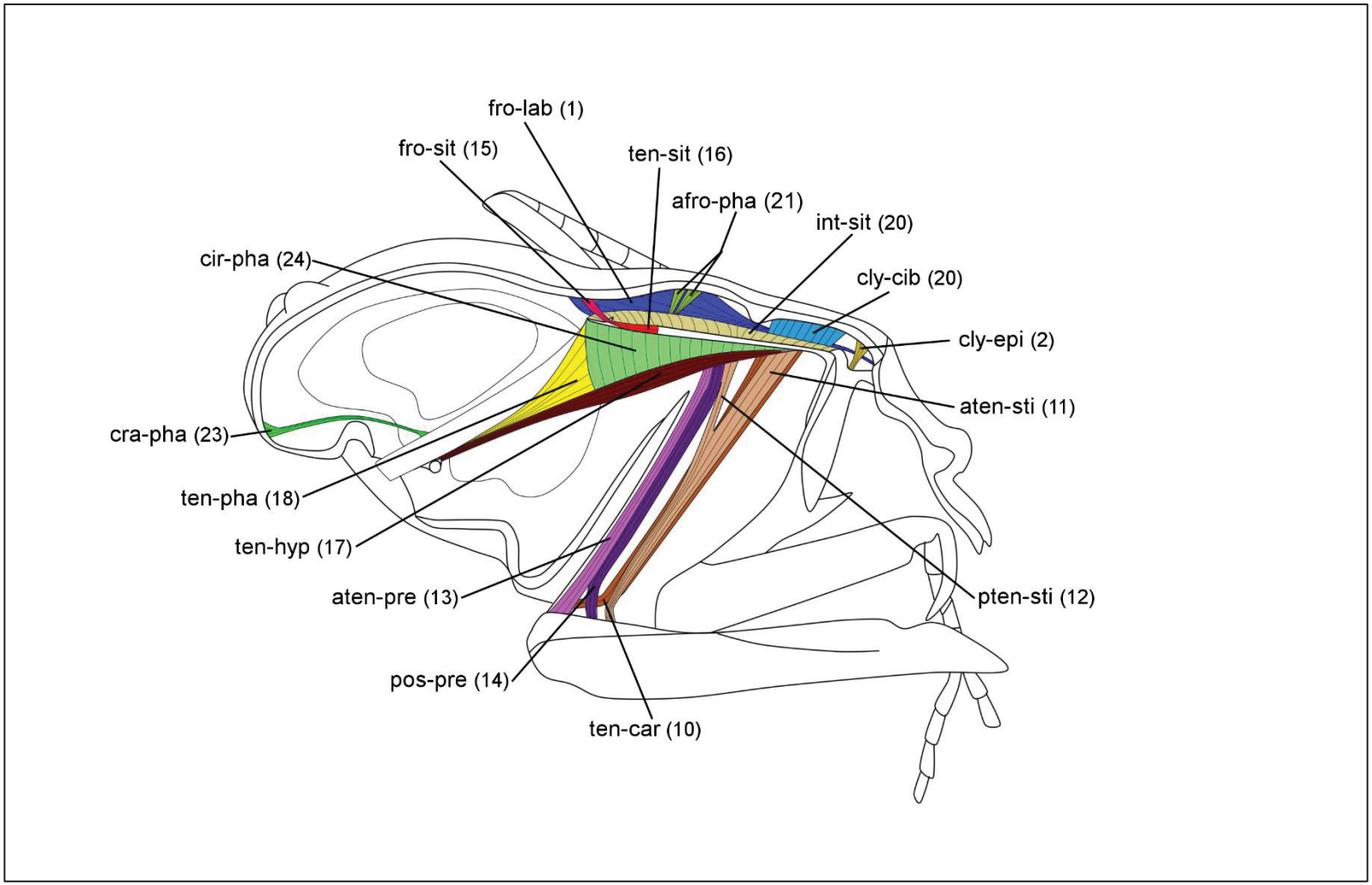

FIGURE 111. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DIALICTUS OPACUS* (MOURE, 1940) (APIDAE: HALICTINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

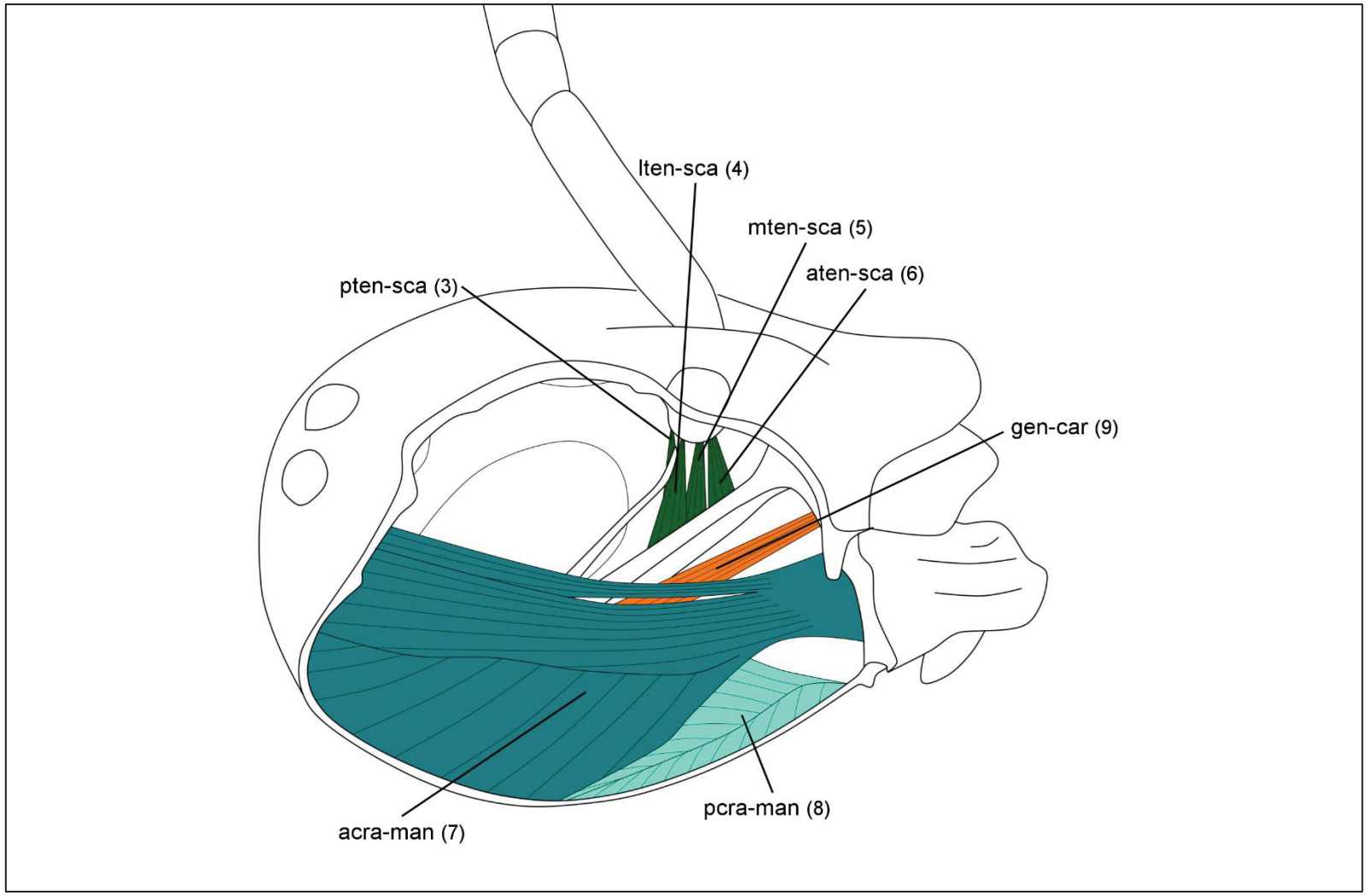

FIGURE 112. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DUFOUREA* SP. (APIDAE: HALICTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

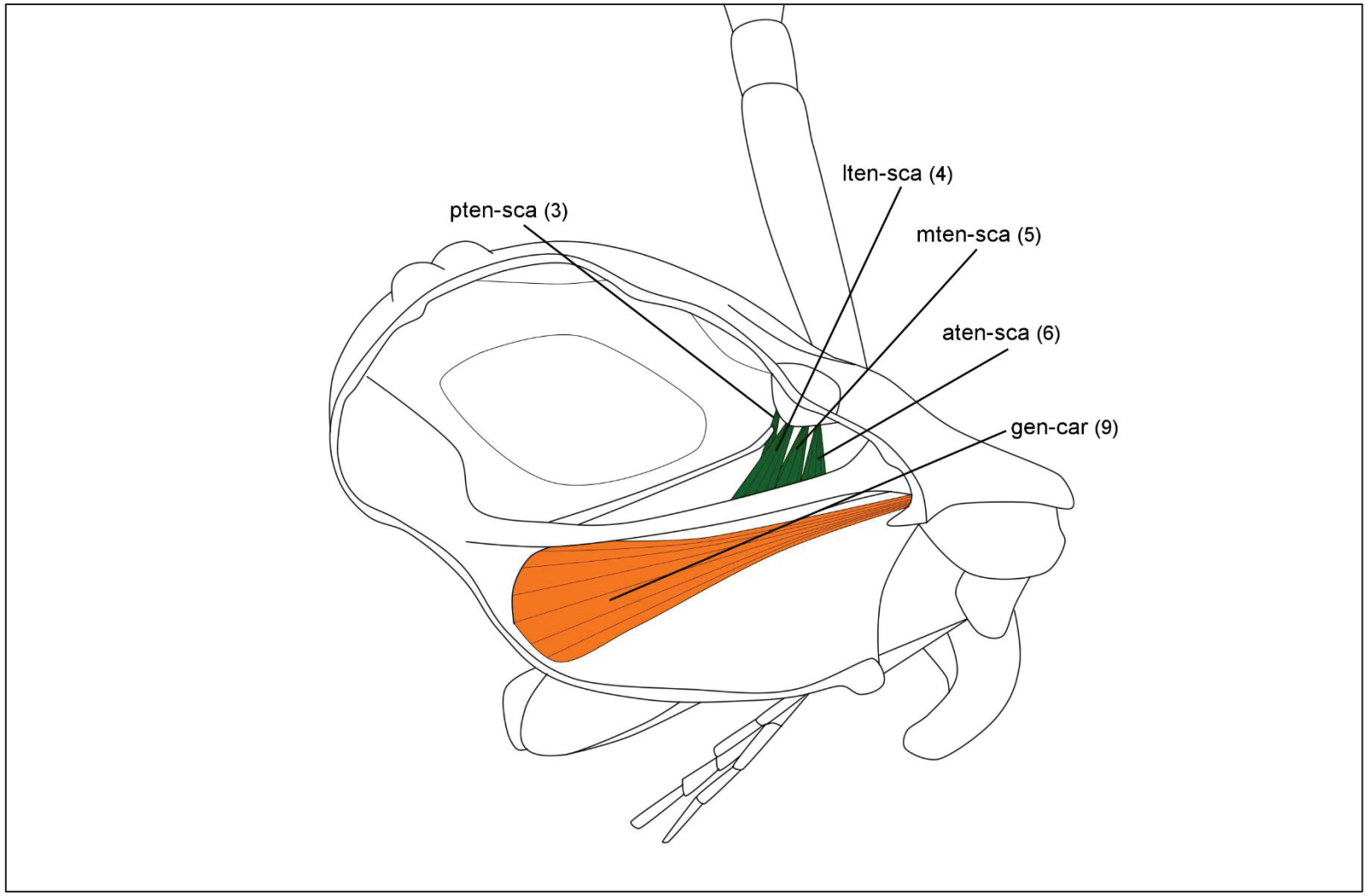

FIGURE 113. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DUFOUREA* SP. (APIDAE: HALICTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

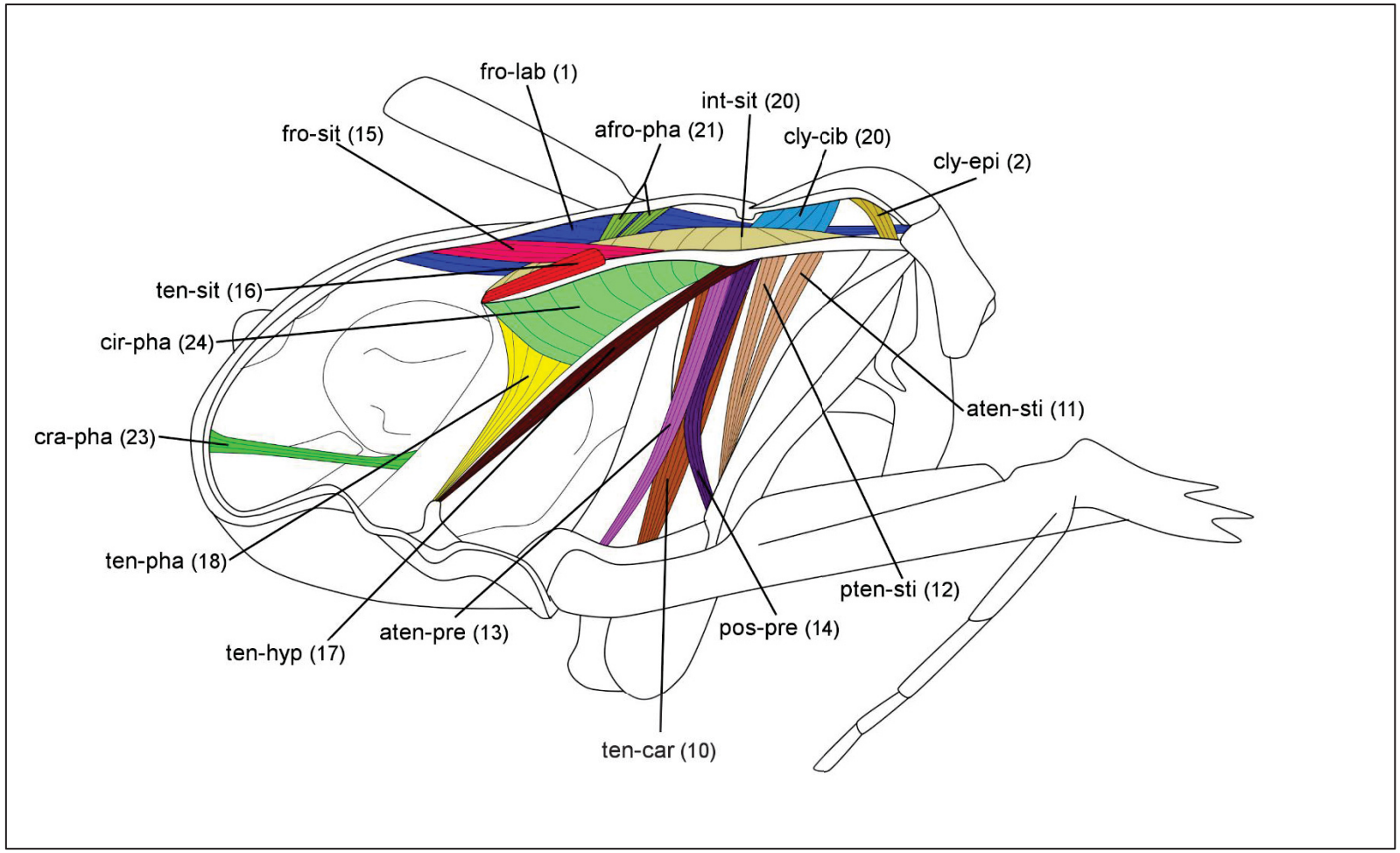

FIGURE 114. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *DUFOUREA* SP. (APIDAE: HALICTINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

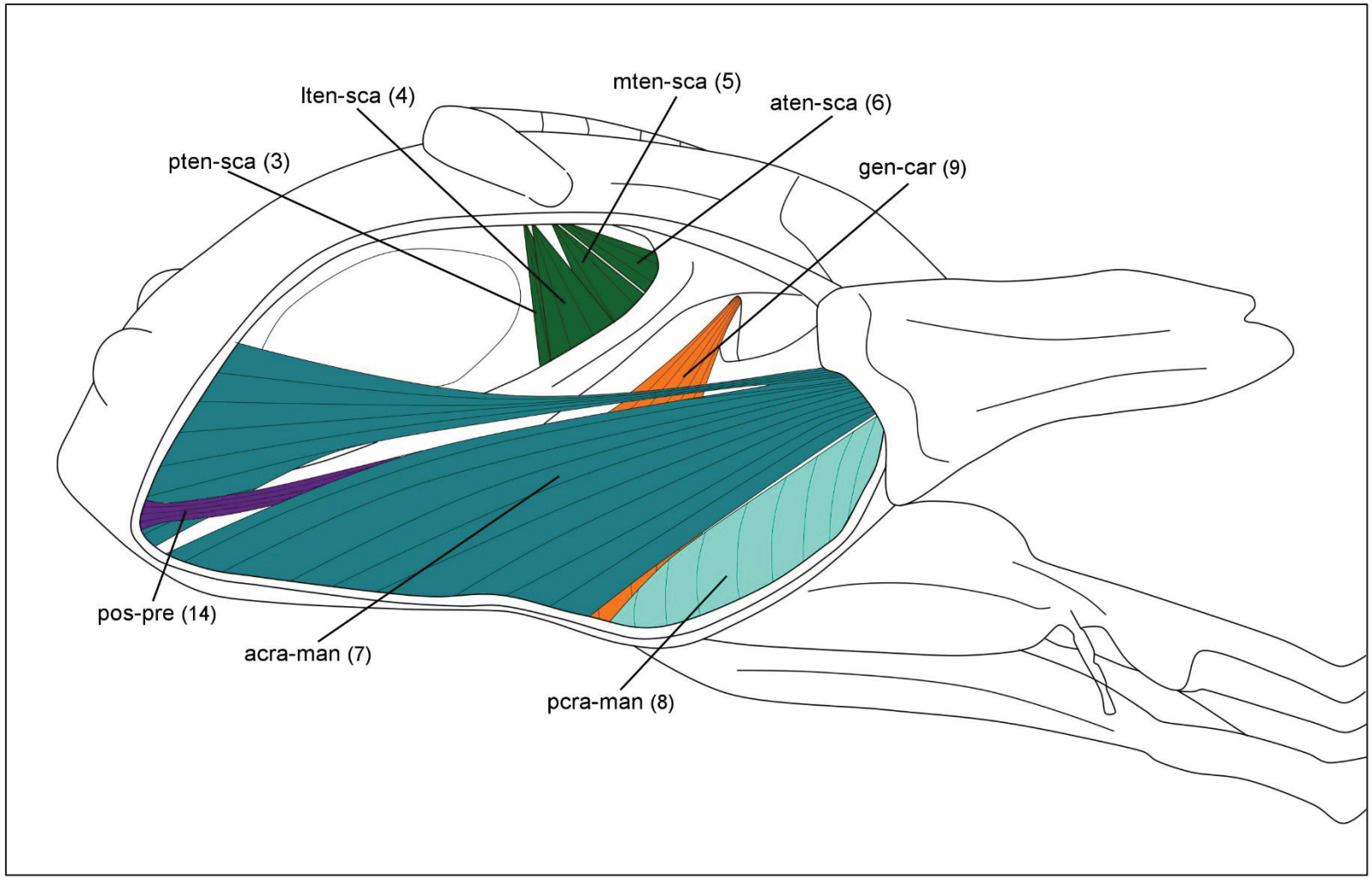

FIGURE 115. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MOUREANTHIDIUM PARANAENSE* URBAN, 1995 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

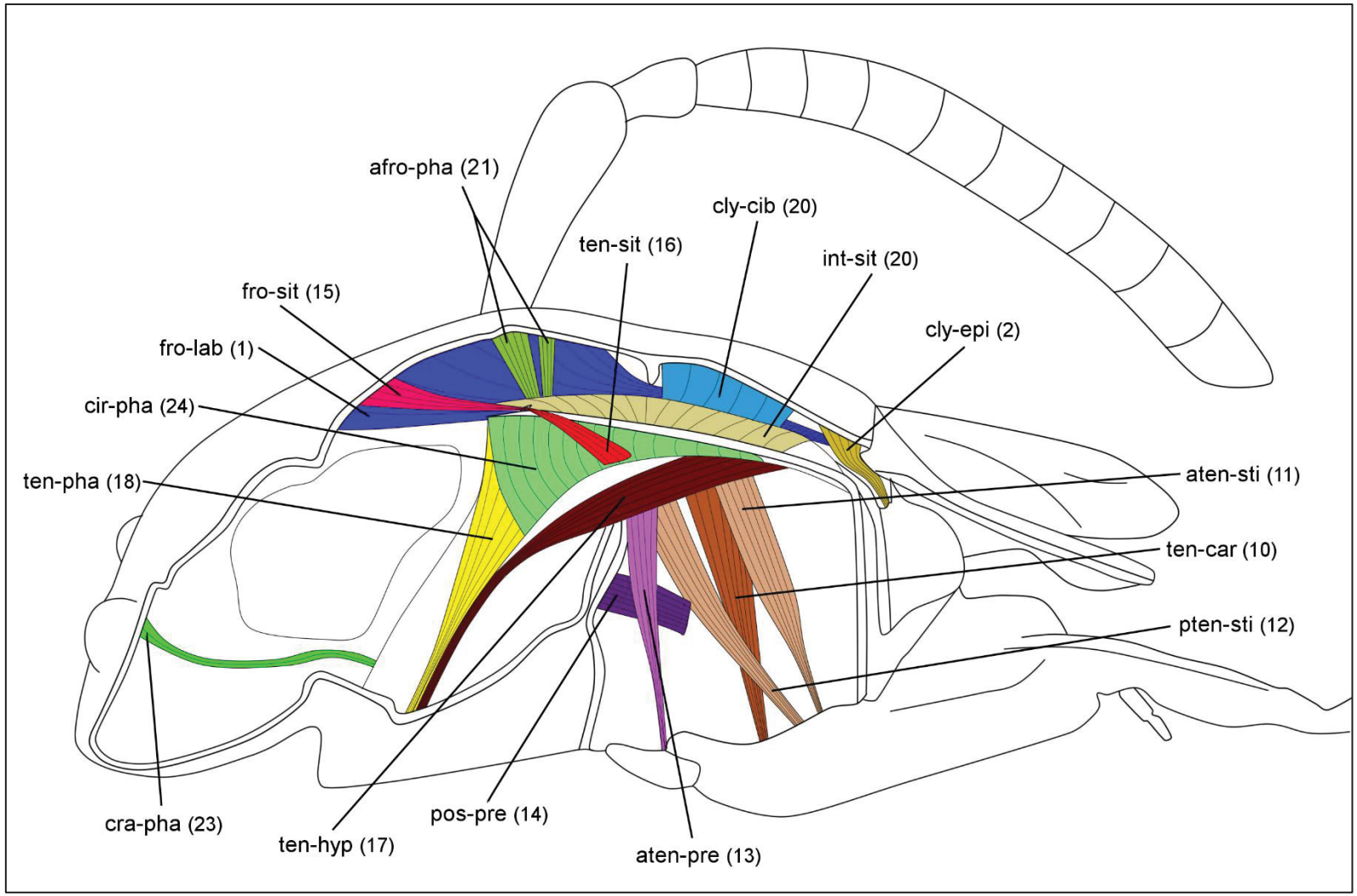

FIGURE 116. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MOUREANTHIDIUM PARANAENSE* URBAN, 1995 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

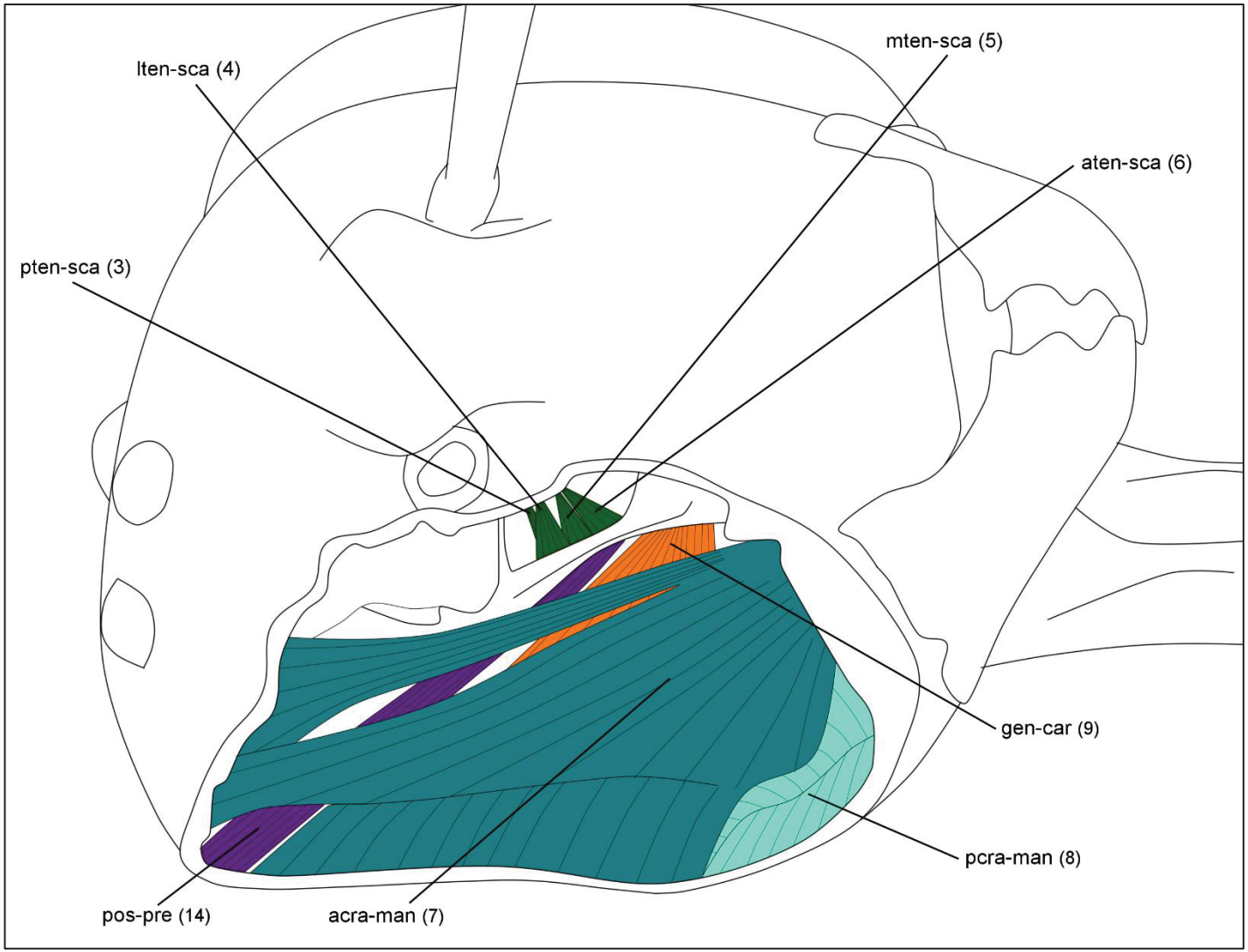

FIGURE 117. EXTRINSIC MUSCLES OF THE HEAD OF MALE *MOUREANTHIDIUM PARANAENSE* URBAN, 1995 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

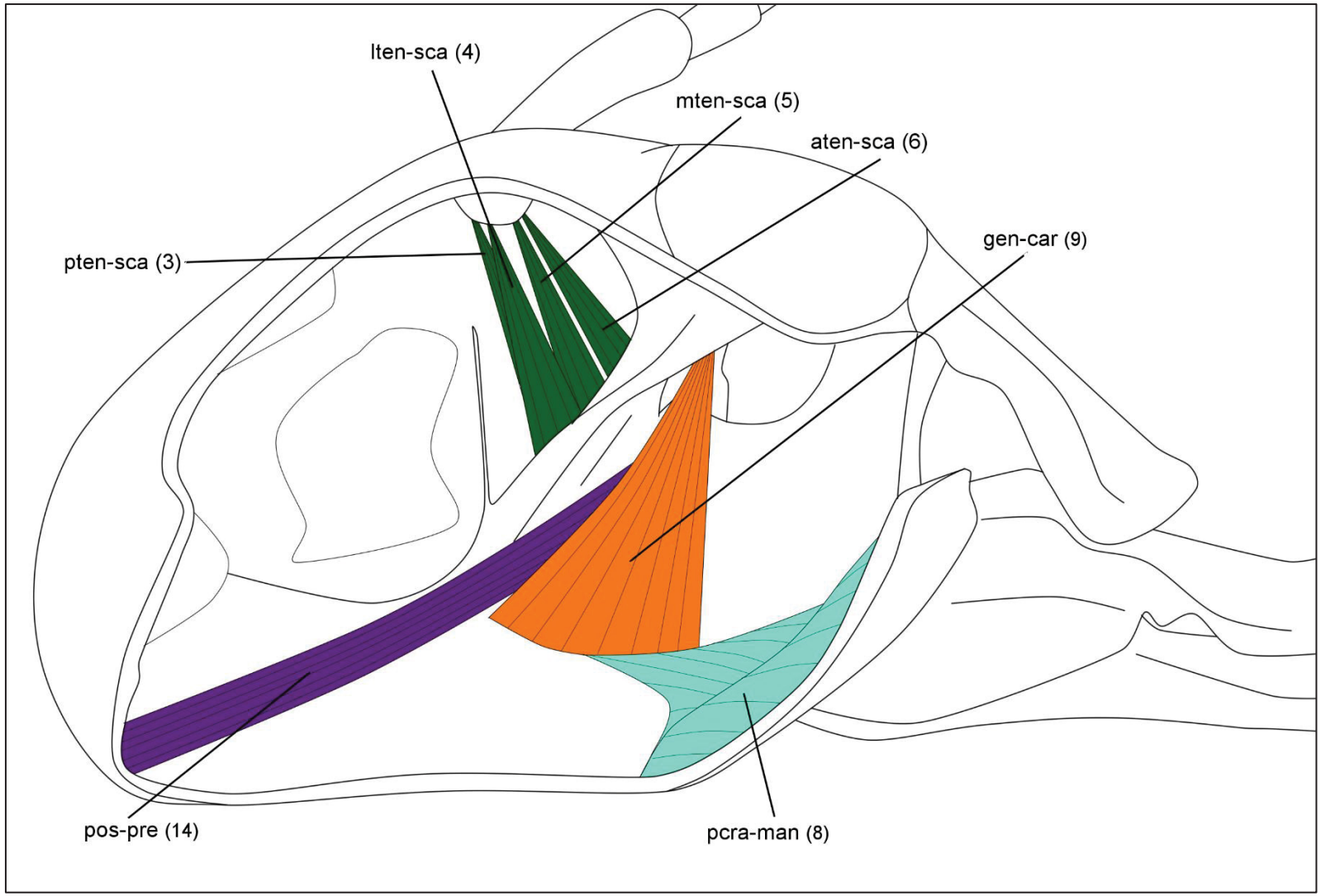

FIGURE 118. EXTRINSIC MUSCLES OF THE HEAD OF MALE *MOUREANTHIDIUM PARANAENSE* URBAN, 1995 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

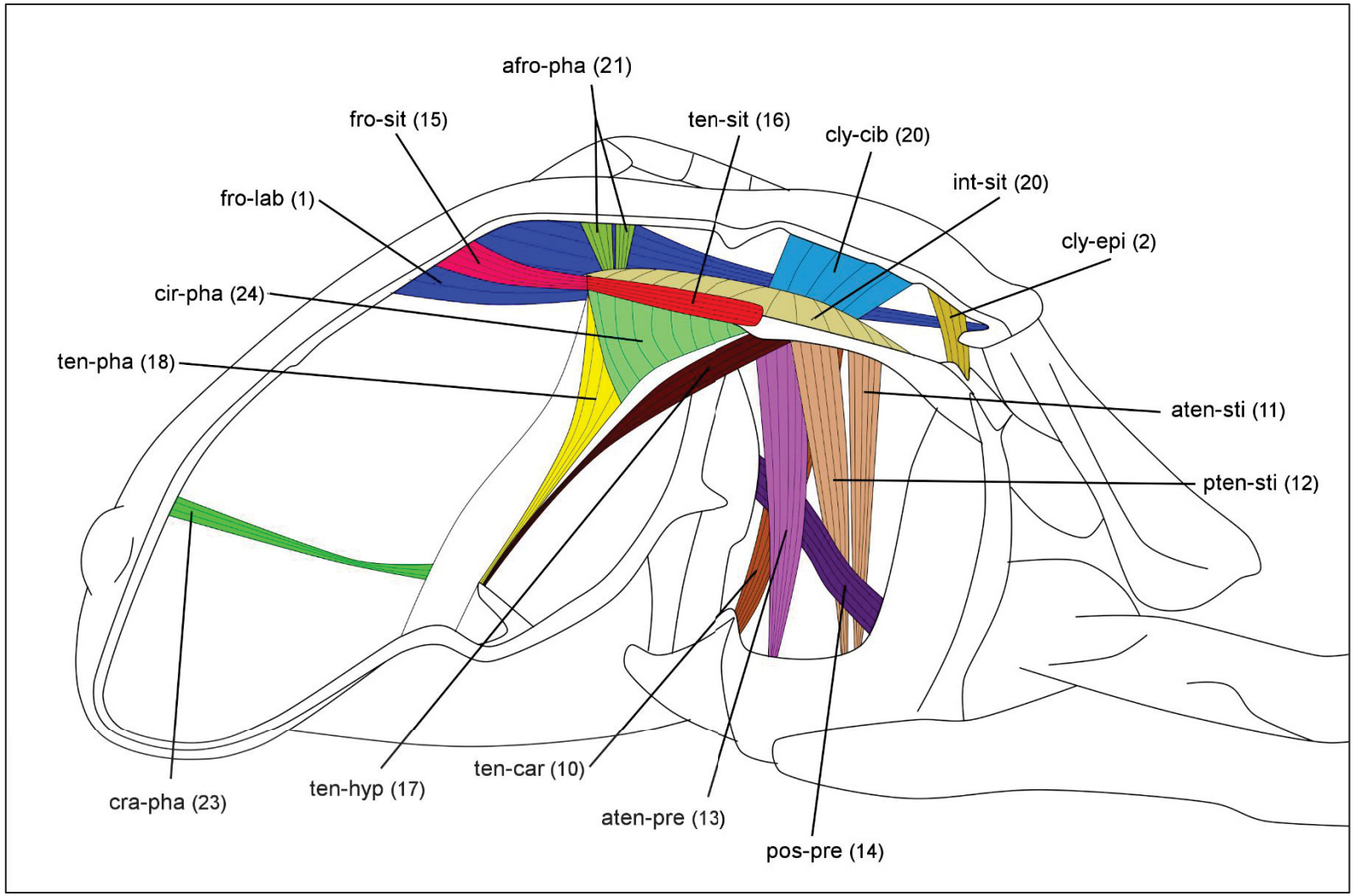

FIGURE 119. EXTRINSIC MUSCLES OF THE HEAD OF MALE *MOUREANTHIDIUM PARANAENSE* URBAN, 1995 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

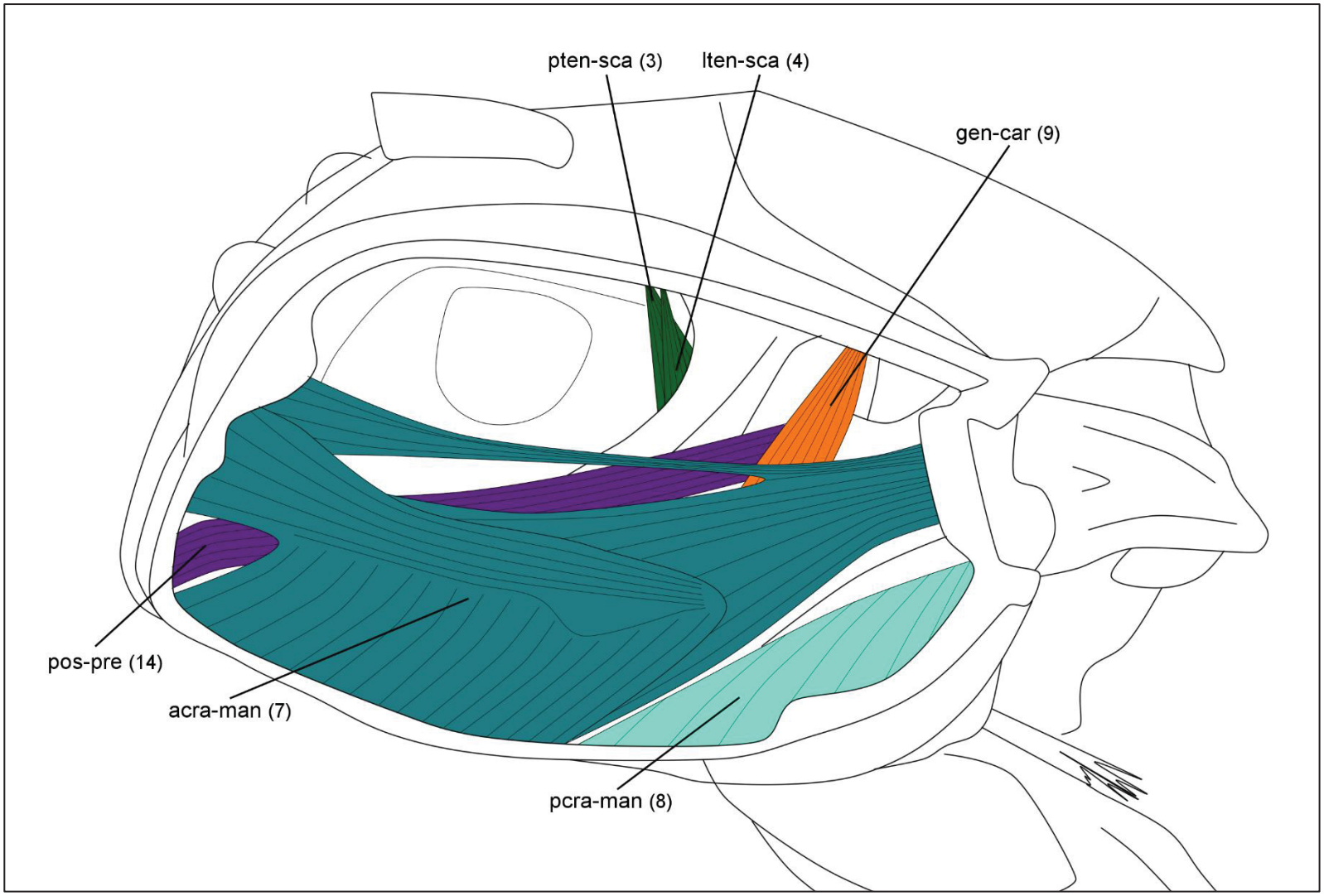

FIGURE 120. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COELIOXYS* SP. (APIDAE: MEGACHILINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

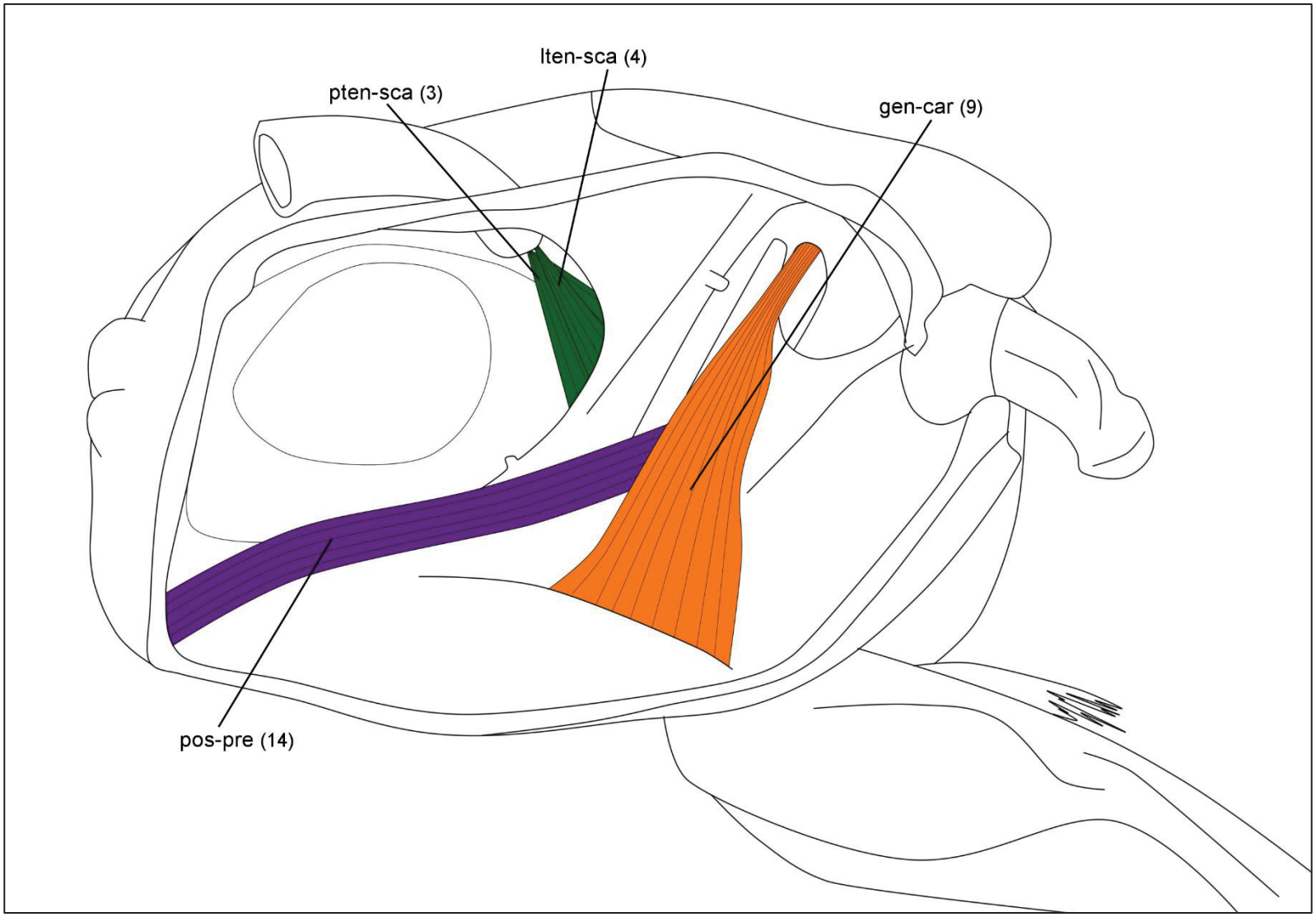

FIGURE 121. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COELIOXYS* SP. (APIDAE: MEGACHILINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

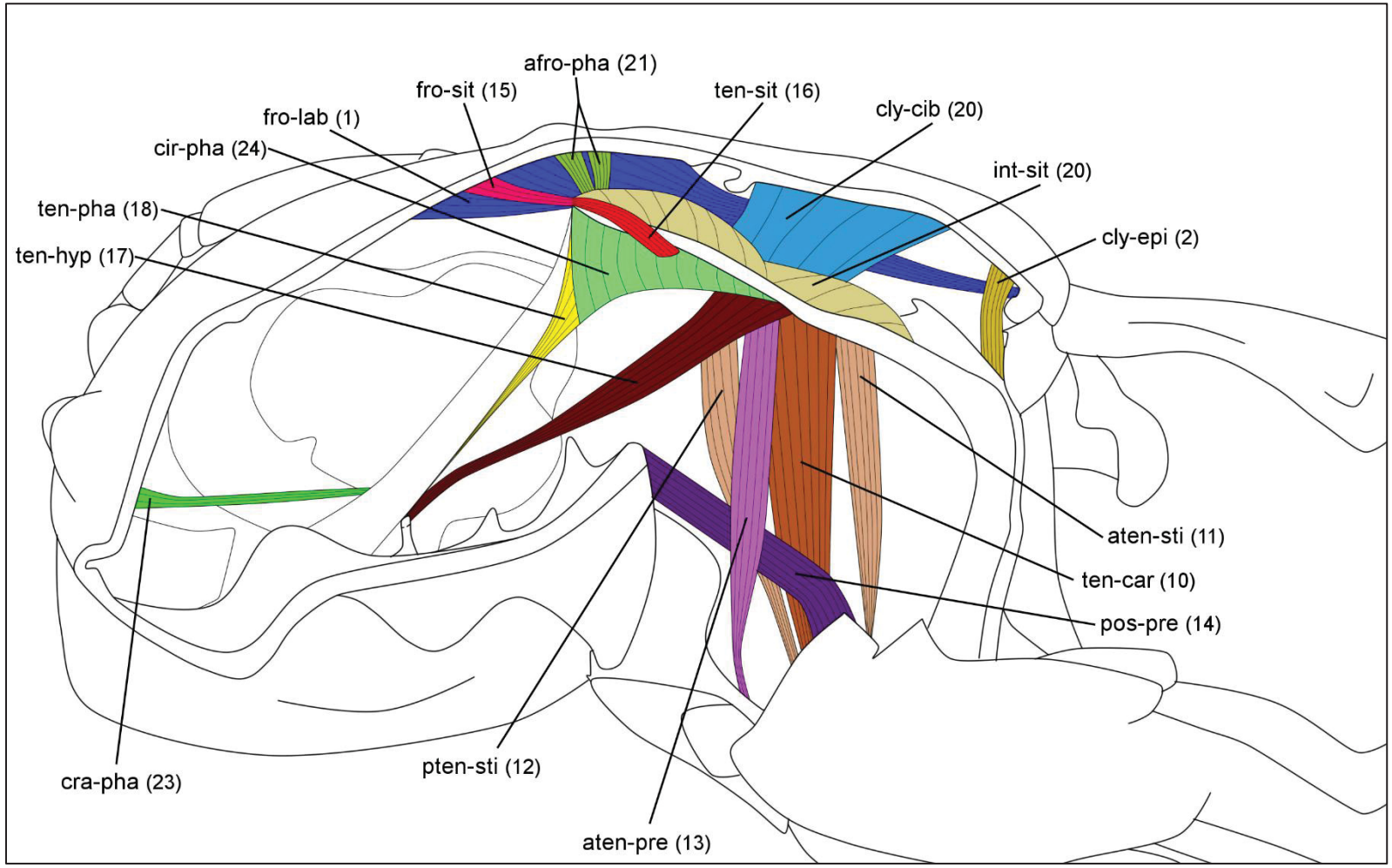

FIGURE 122. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COELIOXYS* SP. (APIDAE: MEGACHILINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

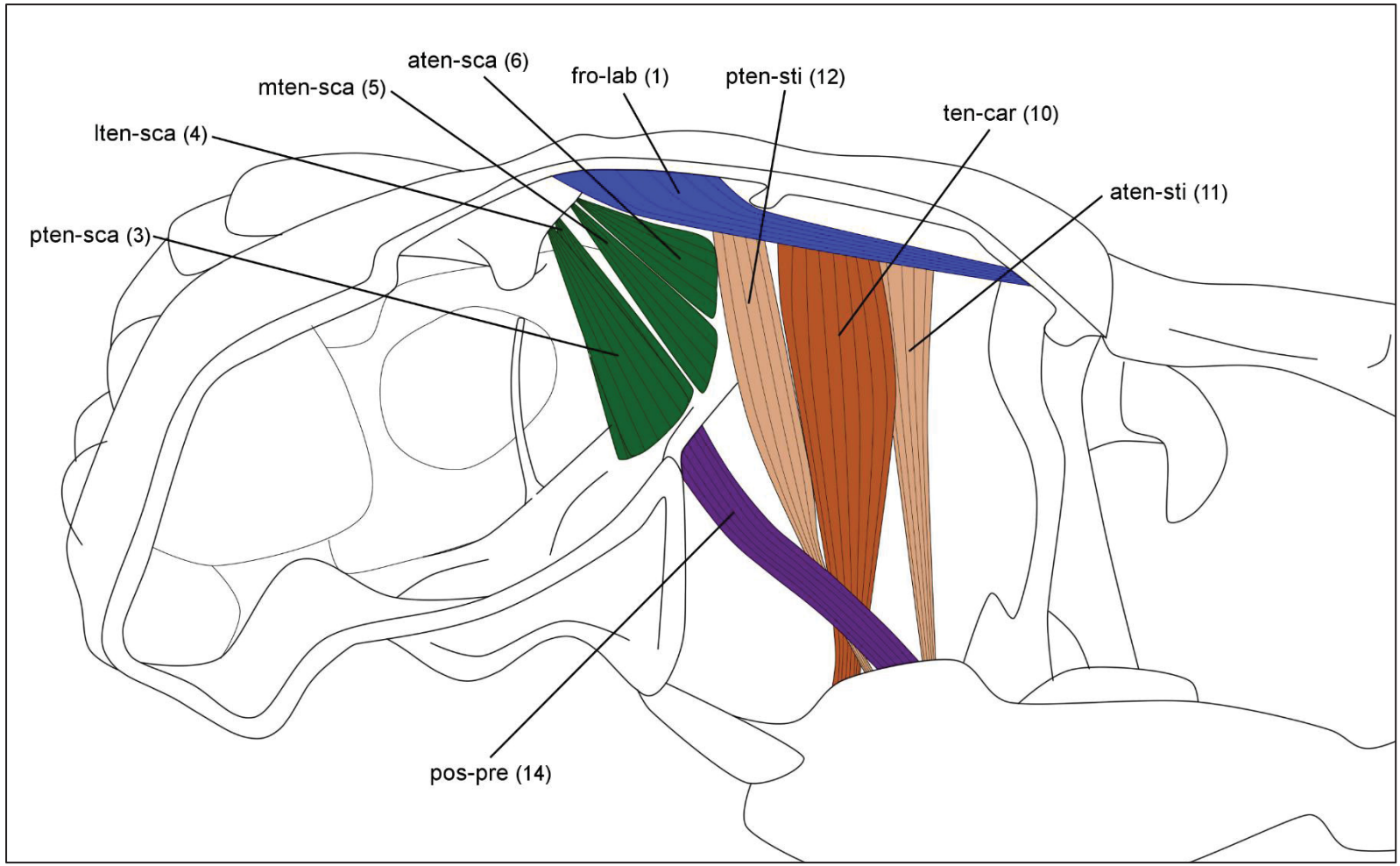

FIGURE 123. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *COELIOXYS* SP. (APIDAE: MEGACHILINAE). SAGGITAL SECTION 4 (S4). LATERAL VIEW.

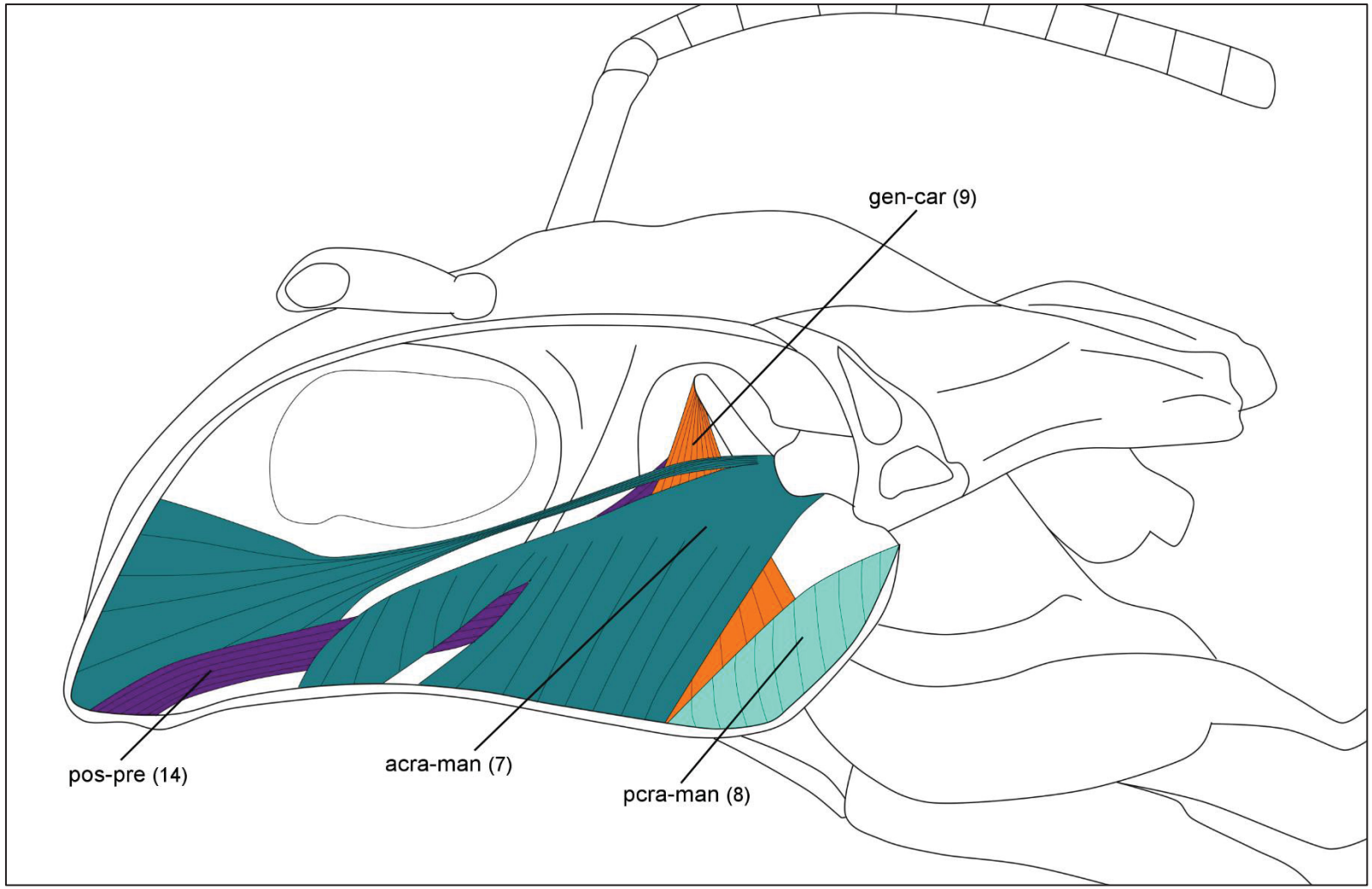

FIGURE 124. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MEGACHILE (MOUREAPIS) APICIPENNIS* SCHROTTKY, 1902 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

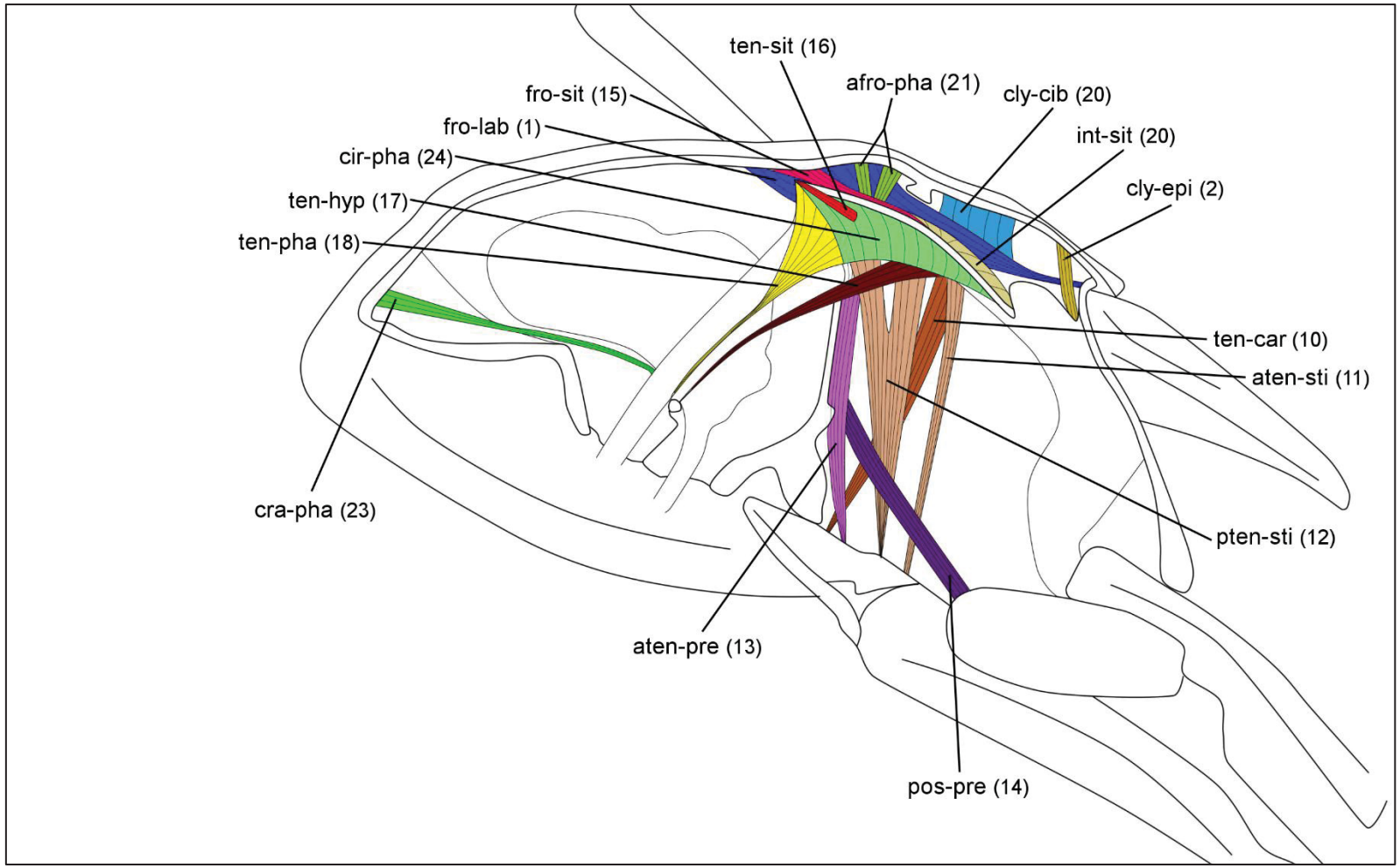

FIGURE 125. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MEGACHILE (MOUREAPIS) APICIPENNIS* SCHROTTKY, 1902 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

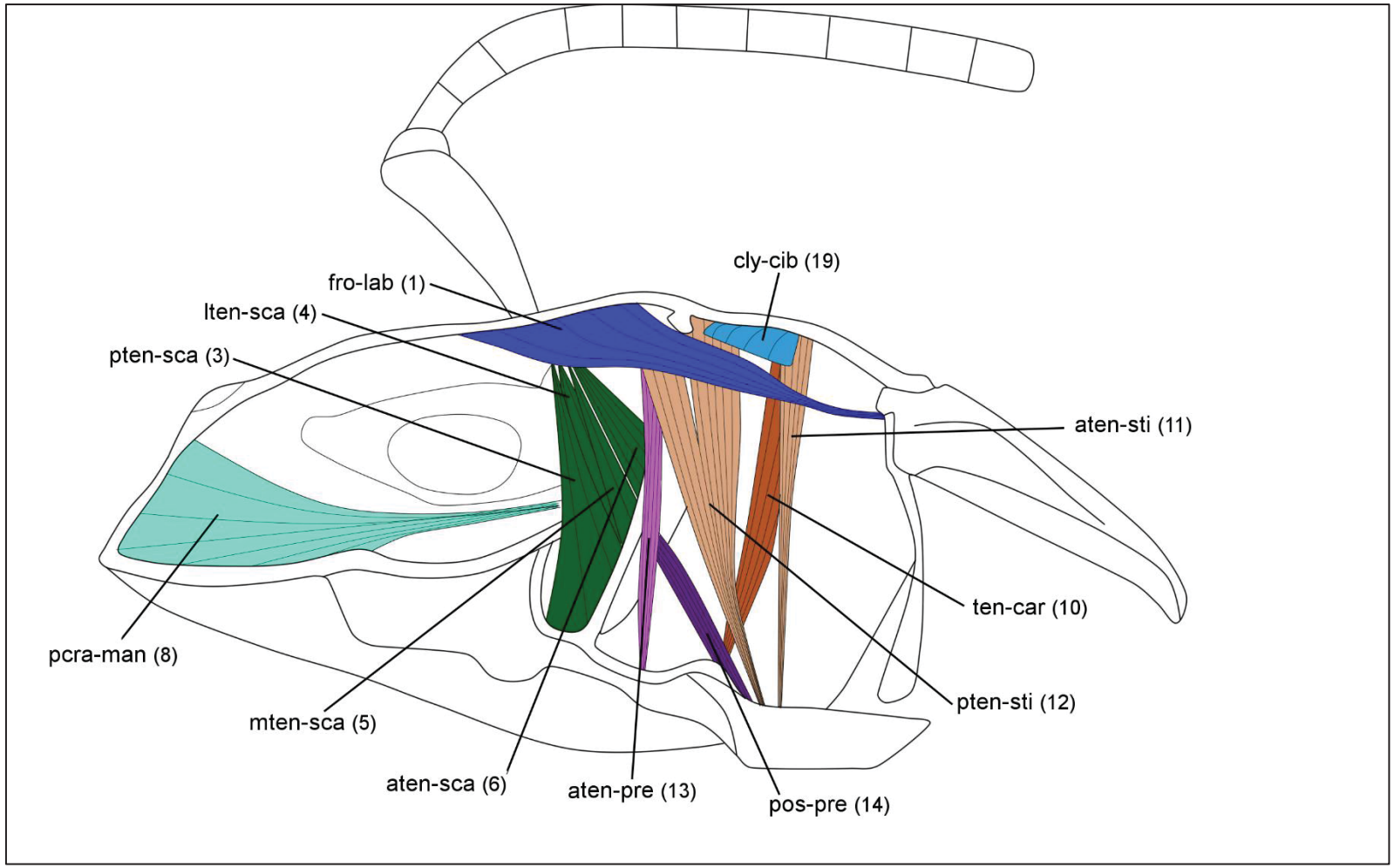

FIGURE 126. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MEGACHILE (MOUREAPIS) APICIPENNIS* SCHROTTKY, 1902 (APIDAE: MEGACHILINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

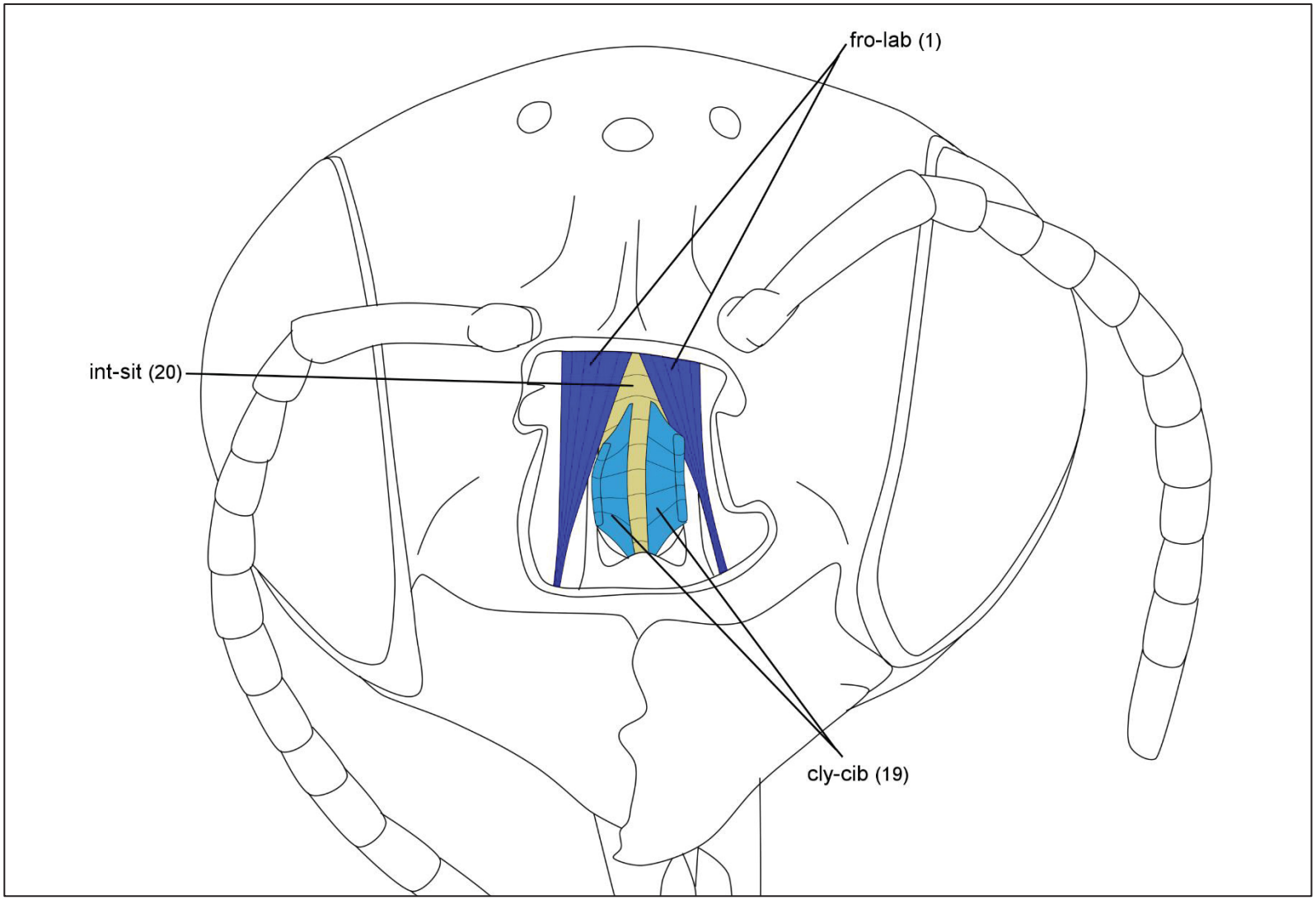

FIGURE 127. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MEGACHILE (MOUREAPIS) APICIPENNIS* SCHROTTKY, 1902 (APIDAE: MEGACHILINAE). CORONAL SECTION 1 (C1). FRONTAL VIEW OF THE CLYPEO-LABRAL AREA.

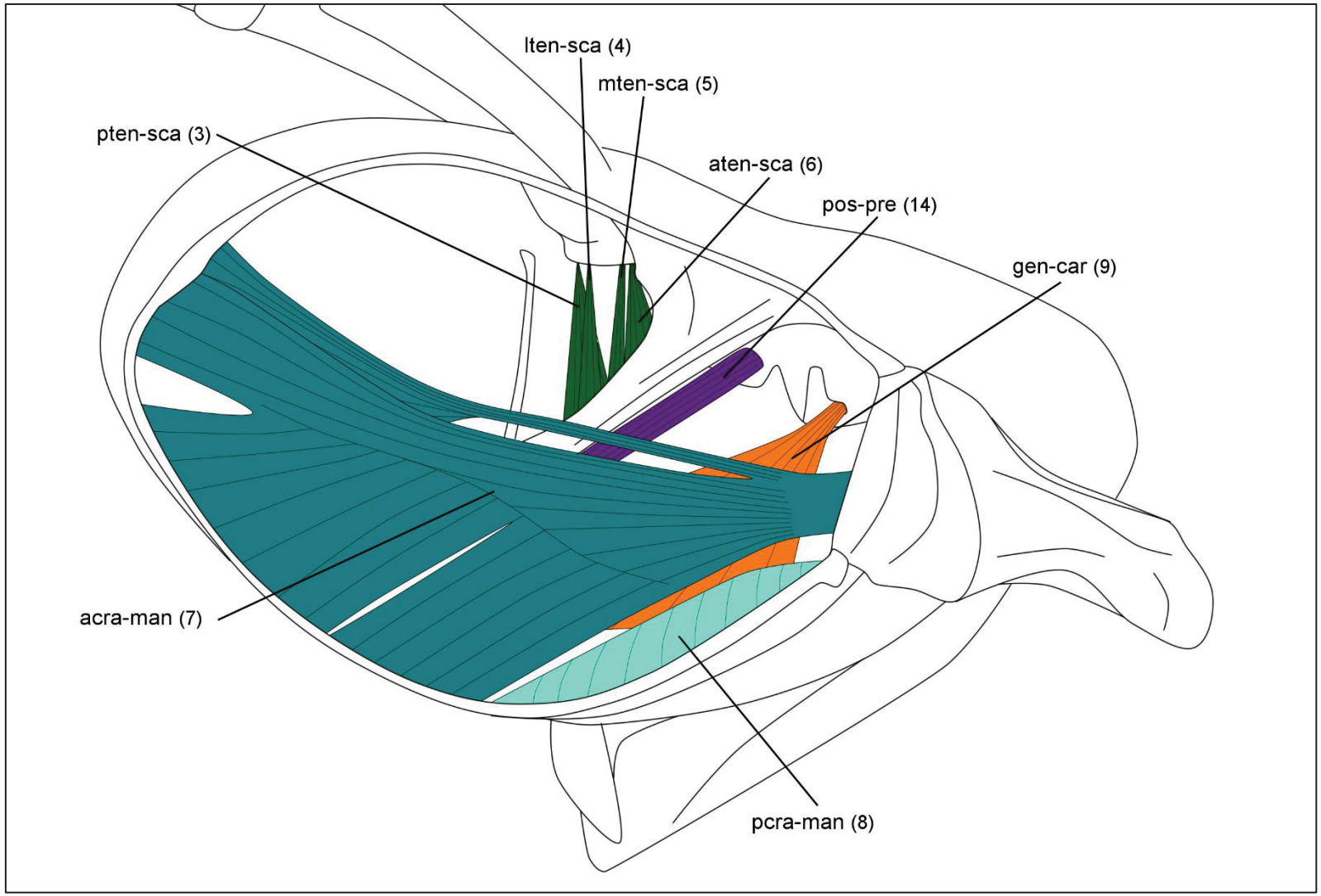

FIGURE 128. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITTA TRICINCTA* KIRBY,1802 (APIDAE: MELITTINAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

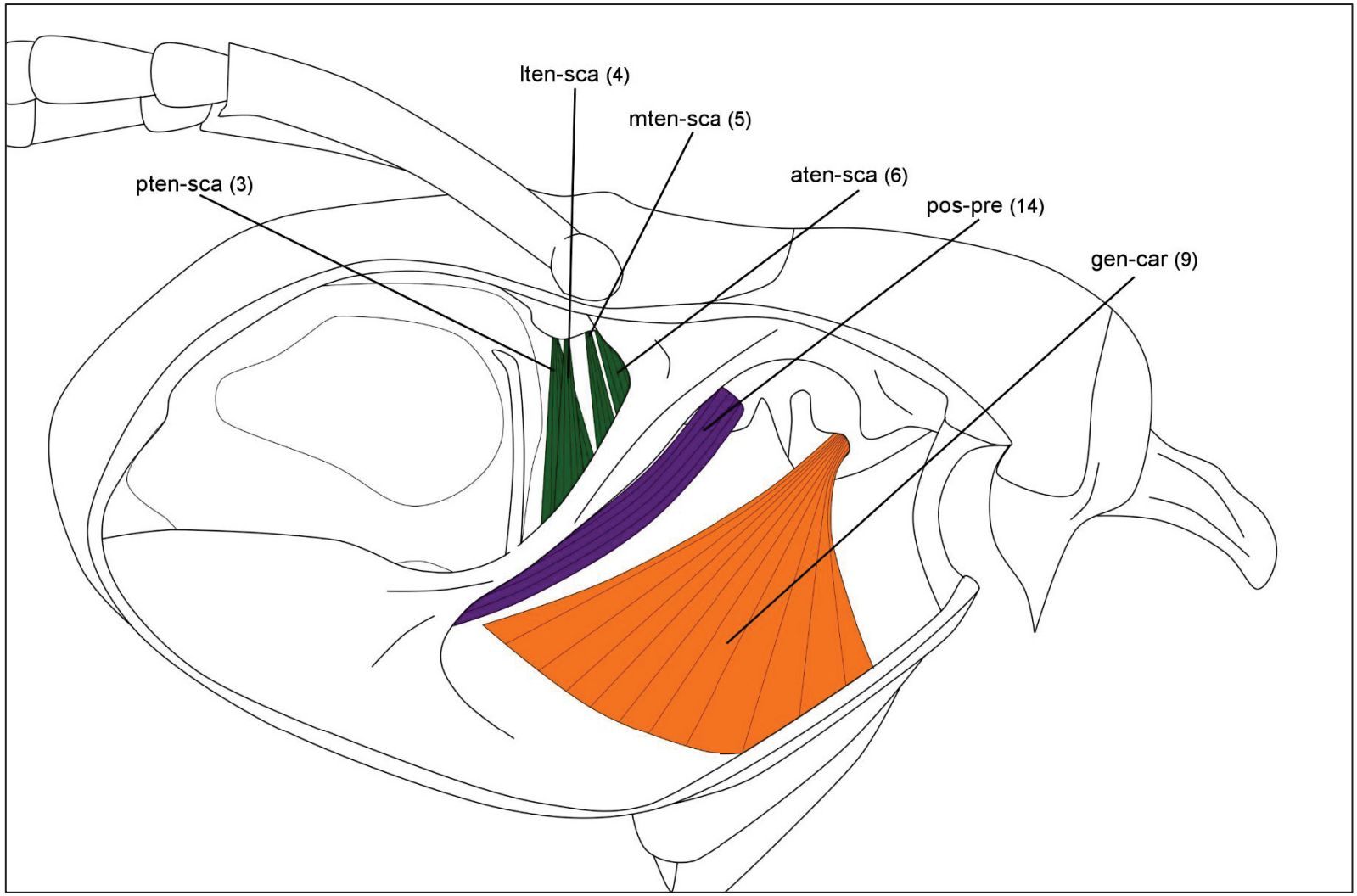

FIGURE 129. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITTA TRICINCTA* KIRBY,1802 (APIDAE: MELITTINAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

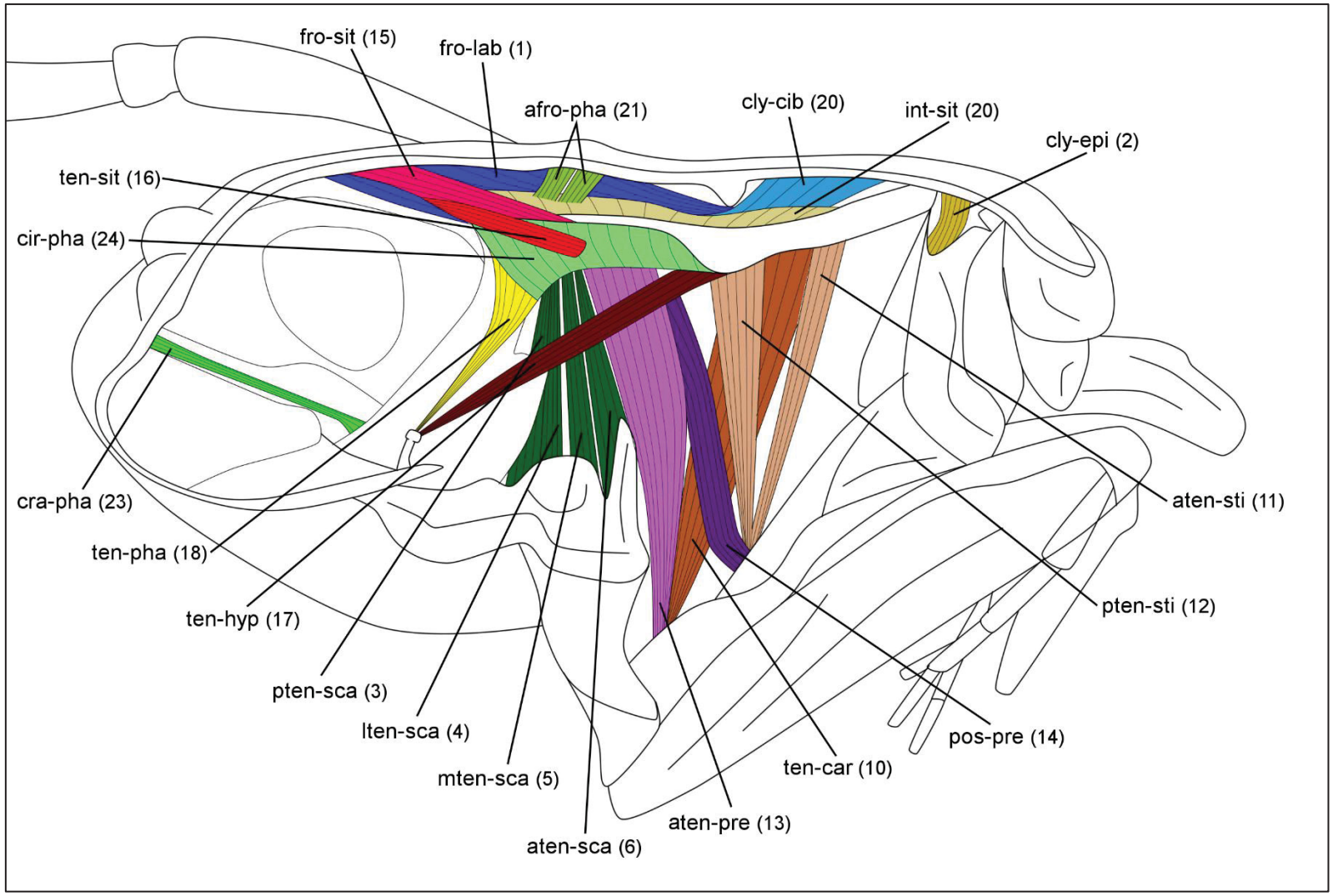

FIGURE 130. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *MELITTA TRICINCTA* KIRBY,1802 (APIDAE: MELITTINAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

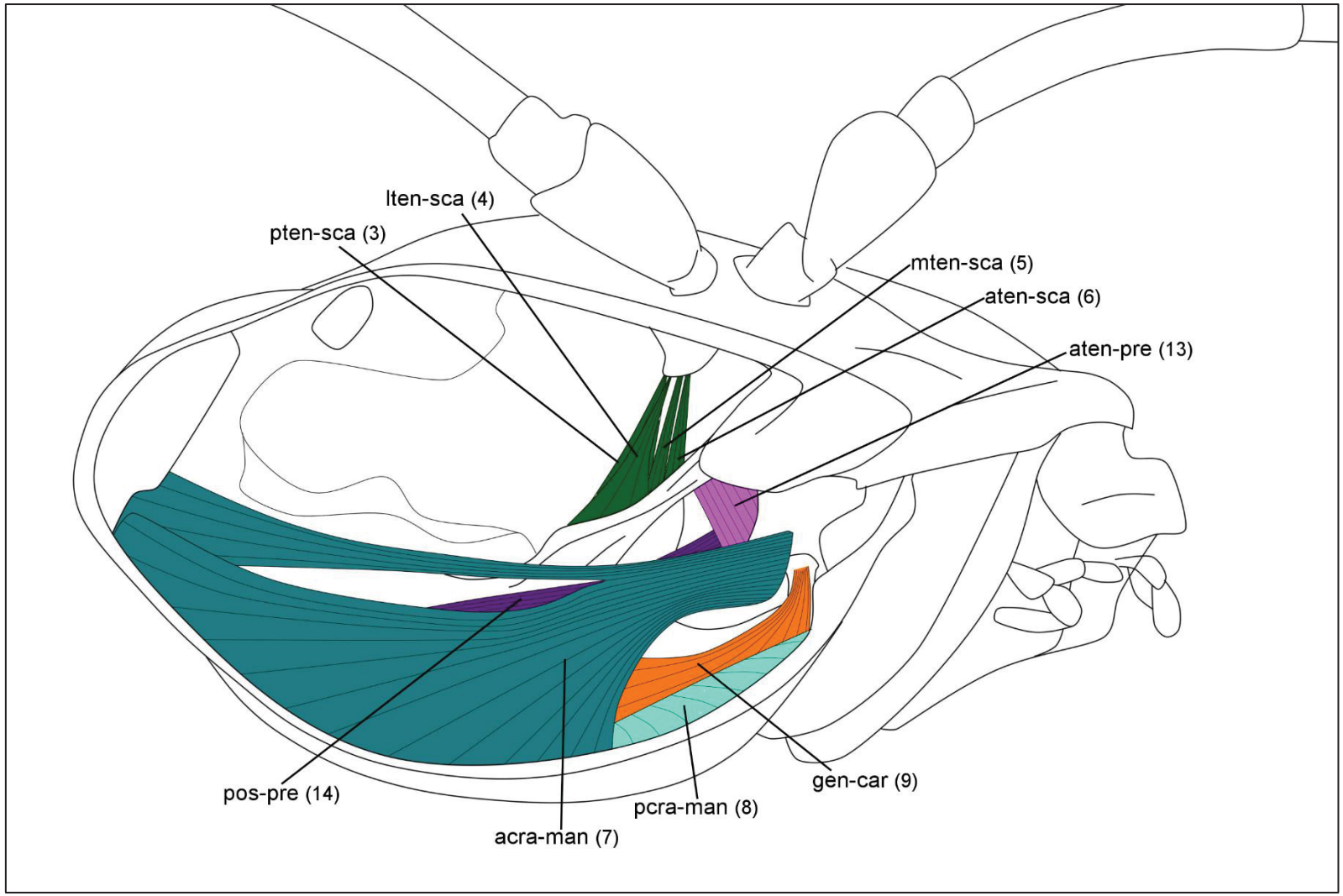

FIGURE 131. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TRYPOXYLON (TRYPARGILUM) LACTITARSE* SAUSSURE, 1867 (HYMENOPTERA: CRABRONIDAE *S.L.*). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

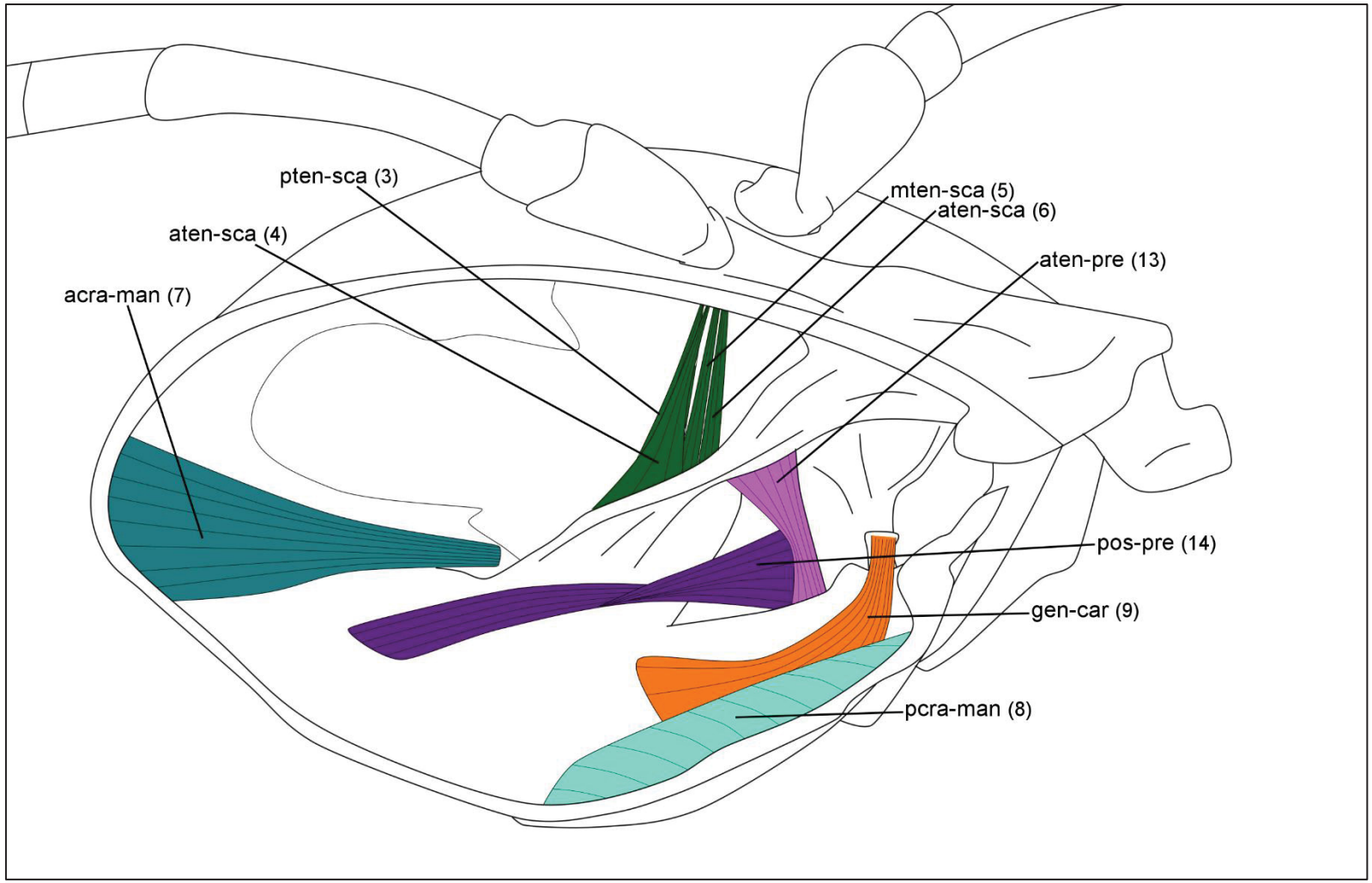

FIGURE 132. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TRYPOXYLON (TRYPARGILUM) LACTITARSE* SAUSSURE, 1867 (HYMENOPTERA: CRABRONIDAE *S.L.*). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

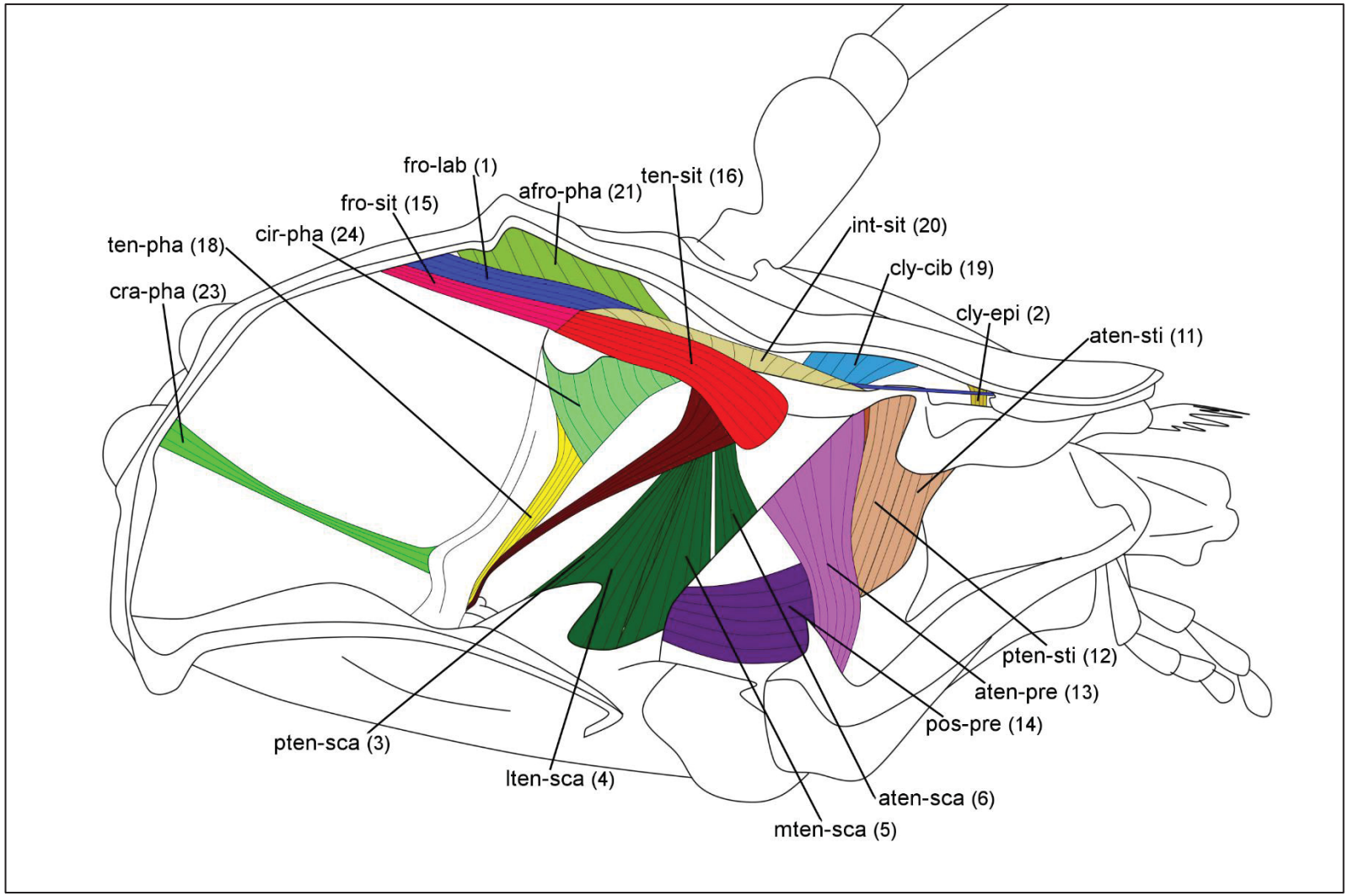

FIGURE 133. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TRYPOXYLON (TRYPARGILUM) LACTITARSE* SAUSSURE, 1867 (HYMENOPTERA: CRABRONIDAE *S.L.*). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

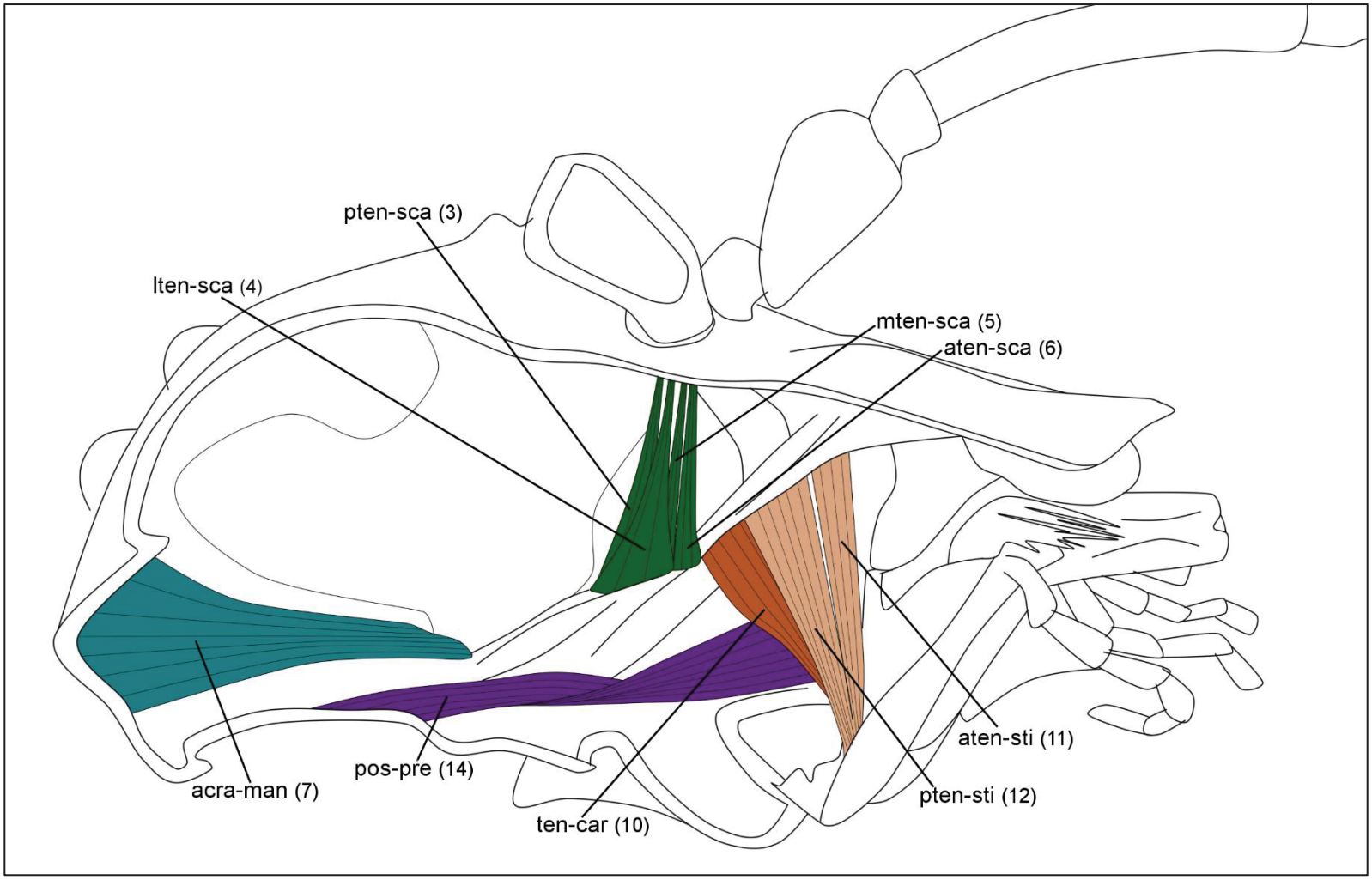

FIGURE 134. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *TRYPOXYLON (TRYPARGILUM) LACTITARSE* SAUSSURE, 1867 (HYMENOPTERA: CRABRONIDAE *S.L.*). SAGGITAL SECTION 4 (S4). LATERAL

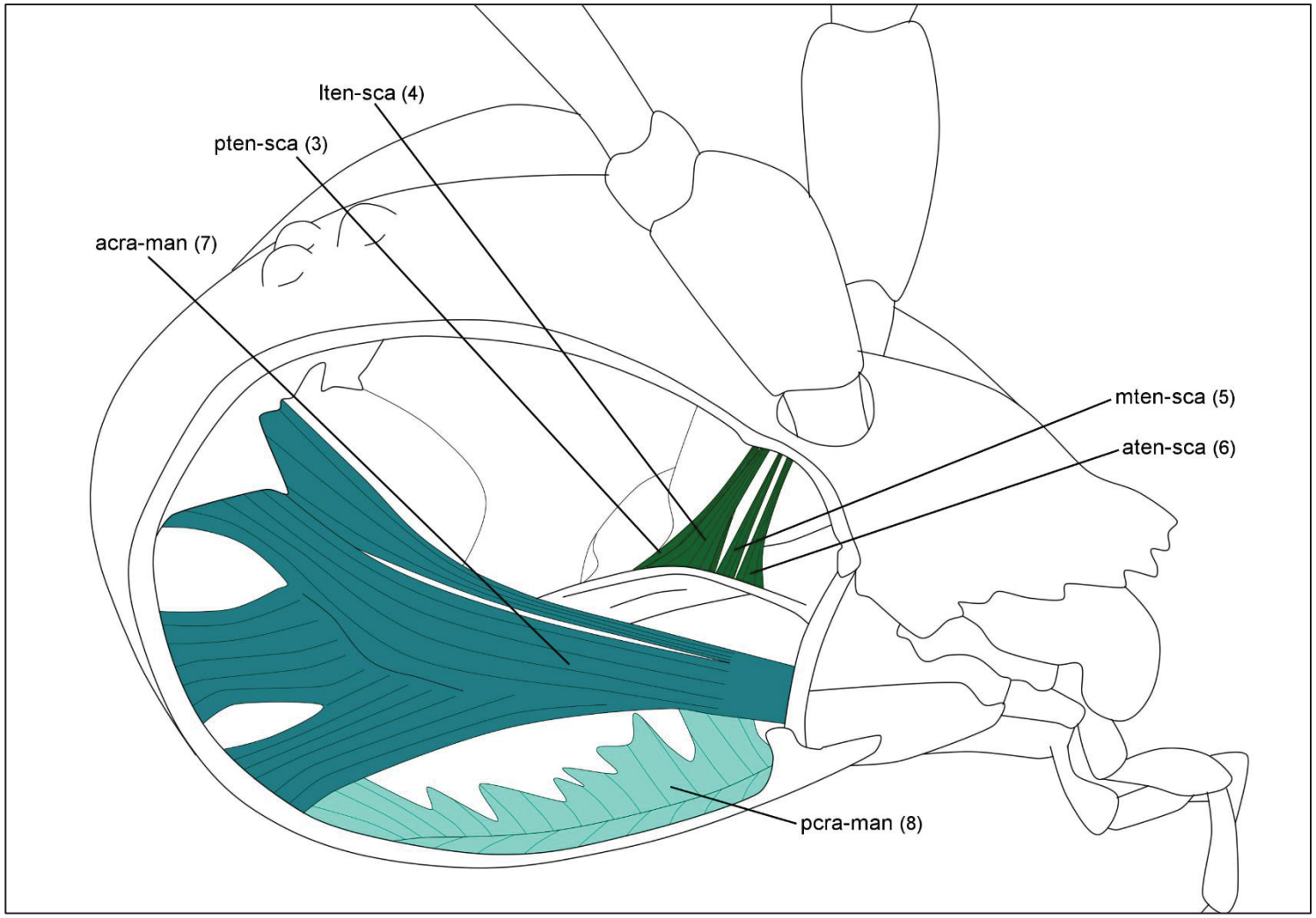

FIGURE 135. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PODIUM* SP. (HYMENOPTERA: SPHECIDAE). SAGGITAL SECTION 1 (S1). LATERAL VIEW.

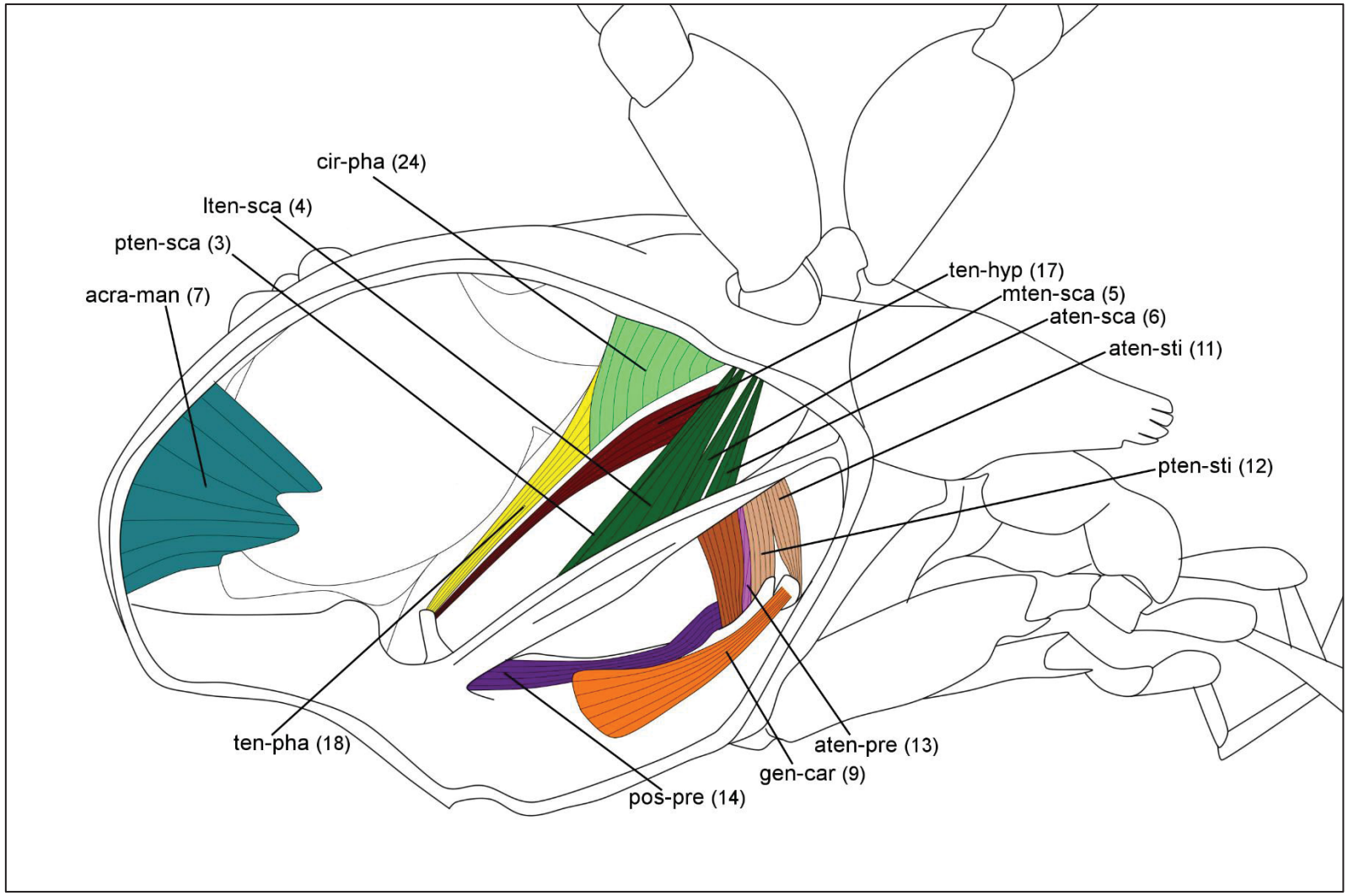

FIGURE 136. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PODIUM* SP. (HYMENOPTERA: SPHECIDAE). SAGGITAL SECTION 2 (S2). LATERAL VIEW.

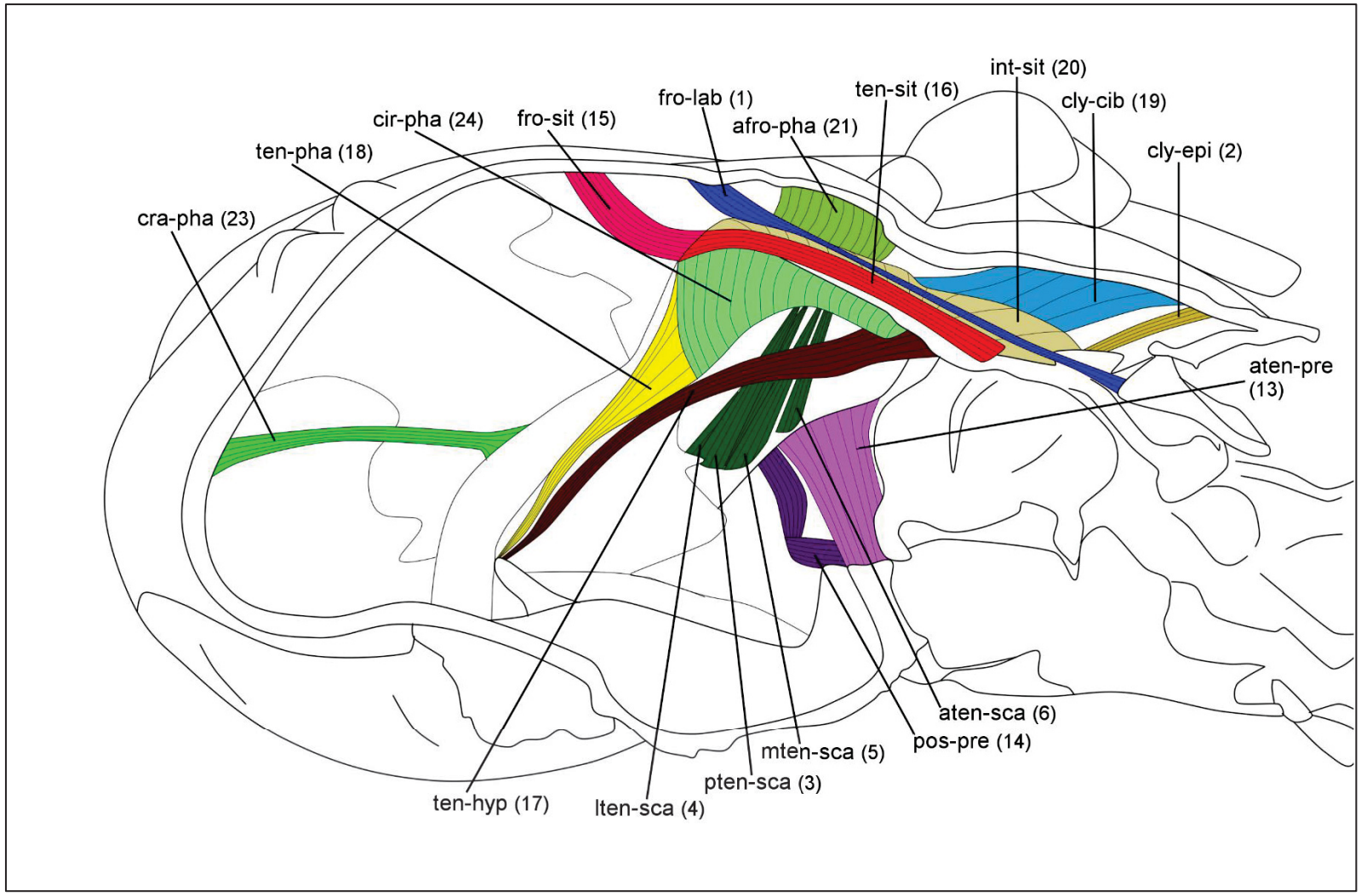

FIGURE 137. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PODIUM* SP. (HYMENOPTERA: SPHECIDAE). SAGGITAL SECTION 3 (S3). LATERAL VIEW.

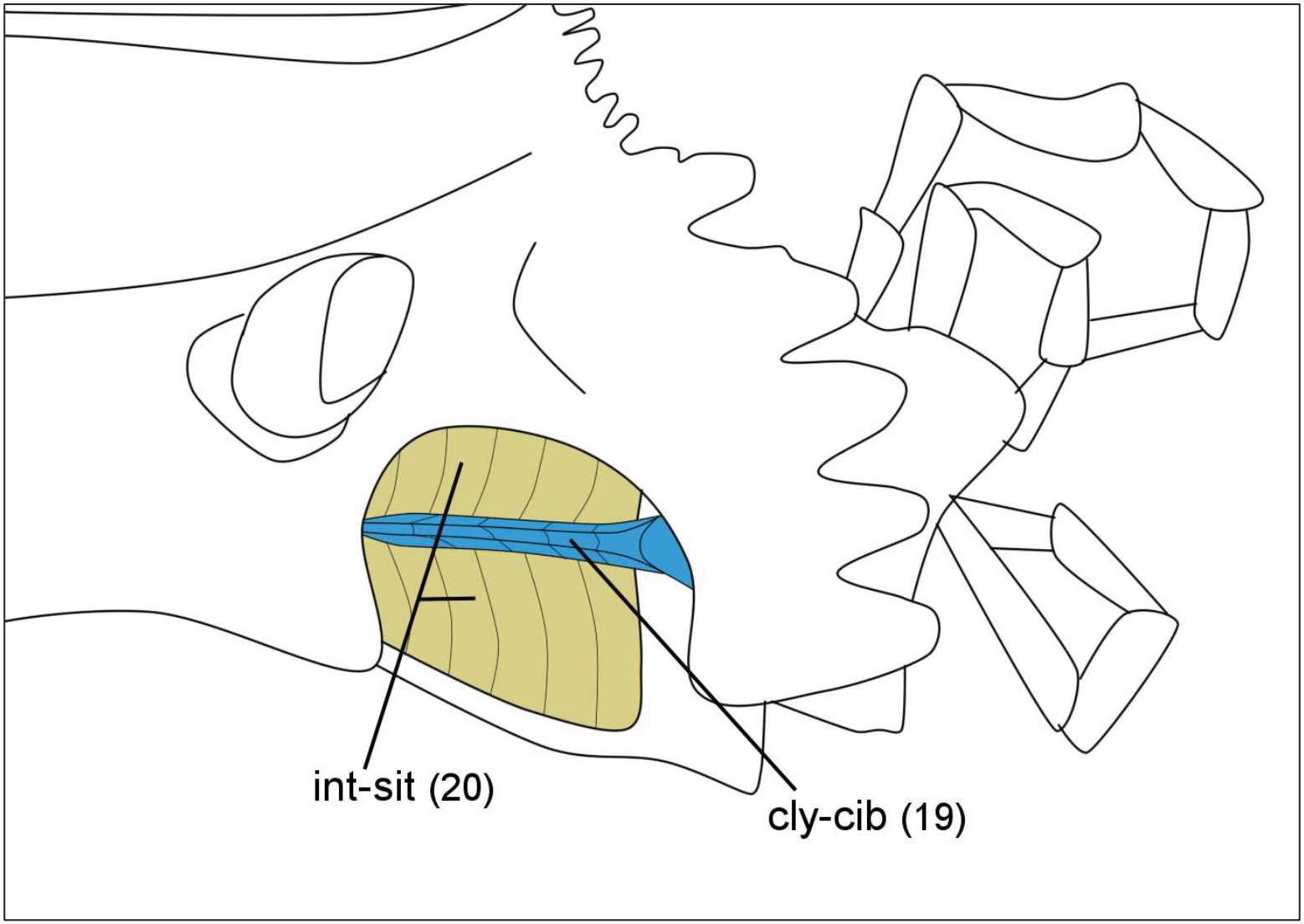

FIGURE 138. EXTRINSIC MUSCLES OF THE HEAD OF FEMALE *PODIUM* SP. (HYMENOPTERA: SPHECIDAE). CORONAL SECTION 1 (C1). FRONTAL VIEW OF THE CLYPEO-LABRAL AREA.

APPENDIX B – ADDITIONAL OPTIMIZATIONS.

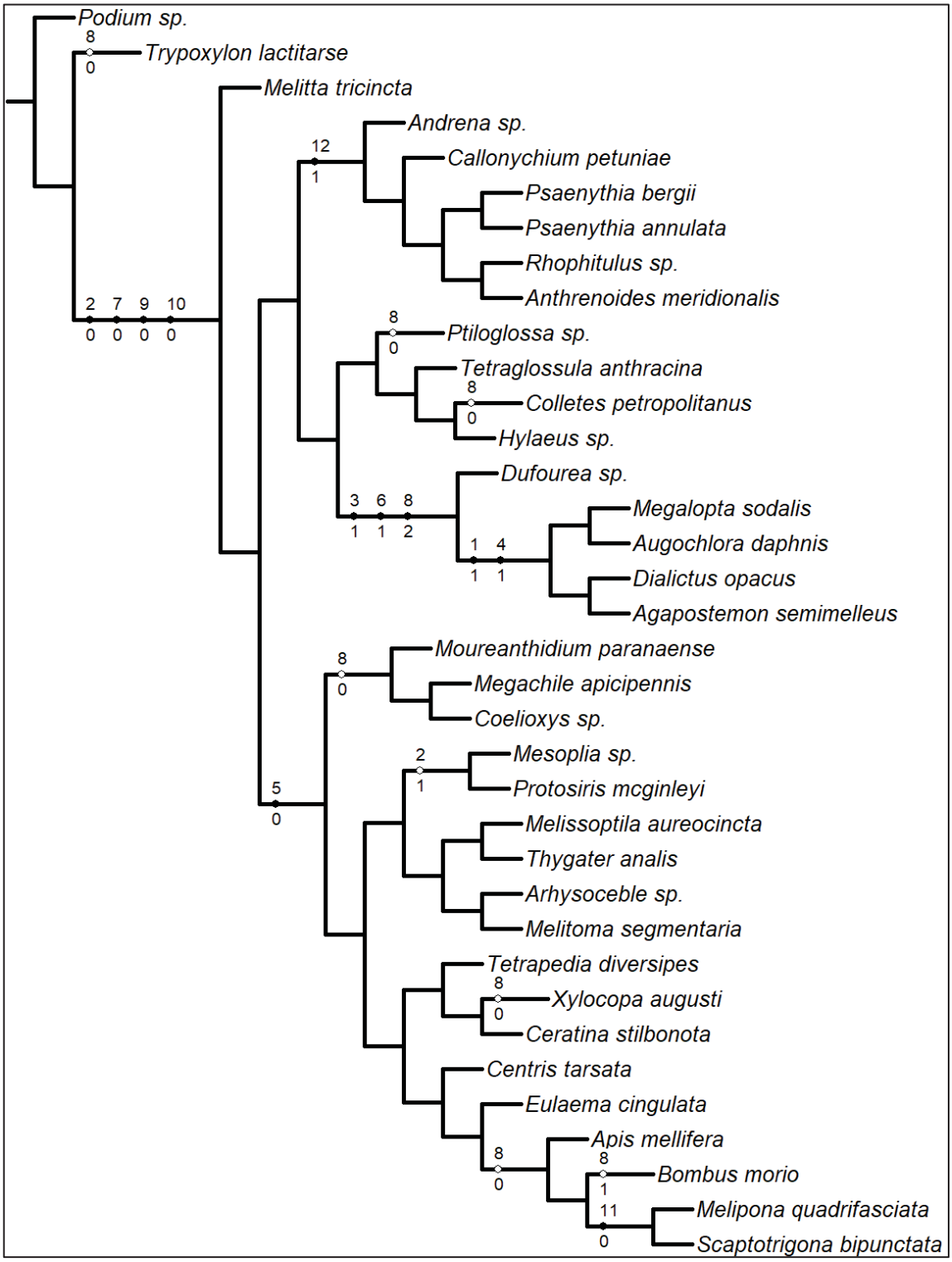

FIGURE 139. ACCTRAN OPTIMIZATION OF HEAD EXTRINSIC MUSCULATURE CHARACTERS ON A SUMMARY TREE FROM HEDTKE ET AL. (2013). THE CHARACTER STATES (BELOW) AND NUMBERS (ABOVE) REFER TO THE CHARACTER DESCRIPTIONS AT THE CHARACTER LIST 5.4. BLACK DOTS INDICATE NONHOMOPLASIOUS CHARACTERS AND WHITE REFER TO HOMOPLASIOUS CHARACTERS.

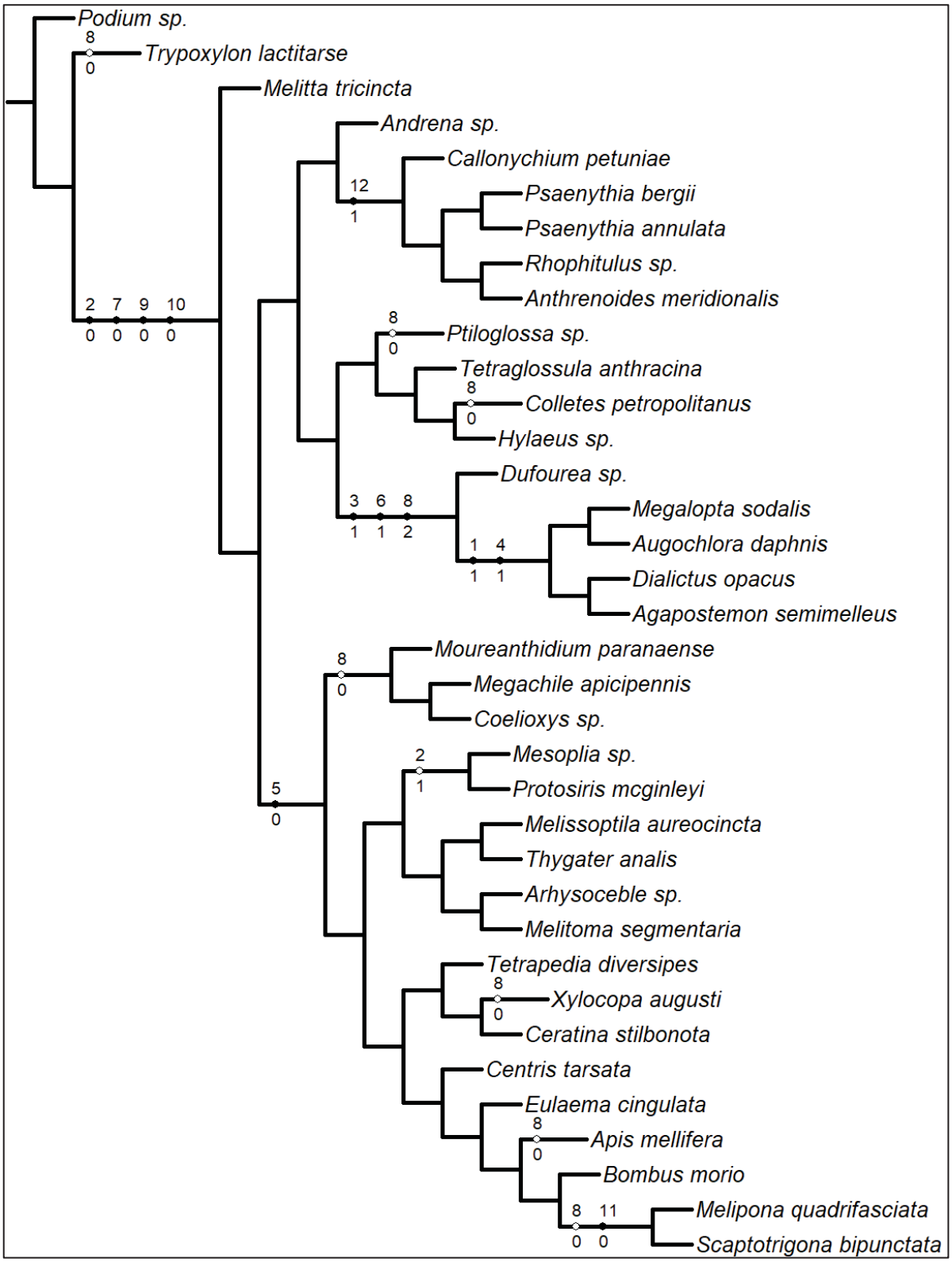

FIGURE 140. DELTRAN OPTIMIZATION OF HEAD EXTRINSIC MUSCULATURE CHARACTERS ON A SUMMARY TREE FROM HEDTKE ET AL. (2013). THE CHARACTER STATES (BELOW) AND NUMBERS (ABOVE) REFER TO THE CHARACTER DESCRIPTIONS AT THE CHARACTER LIST 5.4. BLACK DOTS INDICATE NONHOMOPLASIOUS CHARACTERS AND WHITE REFER TO HOMOPLASIOUS CHARACTERS.